Impact of enzymatic treatment on the flavour-active compounds in lingonberry juice

Master thesis in Technology

University of Turku

Department of Biochemistry

Master's Degree Programme in Food Development

December 2018

Antti Karinpoika Nuutinen

The originality of this thesis has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

UIVERSITY OF TURKU
Department of Biochemistry
NUUTINEN, ANTTI KARINPOIKA: Impact of enzymatic treatment on the flavour-active compounds in lingonberry juice

Master's thesis in Technology, 61 p.
Food Development
December 2018
Supervisors M.Sc. Niina Kelanne, Ph.D. Oskar Laaksonen and Prof. Baoru Yang

---

Lingonberry (*Vaccinium vitis-idaea*) is a wild, abundant, easy to pick, and highly nutritious uncultivated berry. Lingonberries are usually consumed as such, or as juices, jams, and purees. Enzymatic treatment is traditionally used to extract higher juice yields and to increase the yields of various compounds, such as anthocyanins and other phenolic compounds, in the juice. The outcome of enzymatic treatment is dependent upon the type of enzyme, concentration, incubation time and temperature.

The aim of this work was to study how different enzymes (pectinases, cellulose, β-glucosidase) impact on the flavour-active compounds of lingonberry juice. Volatile compounds in freshly pressed lingonberry juices were detected using gas chromatography coupled with mass spectrometry. Seven types of lingonberry juice samples were studied, two of which were references without enzyme treatment and five were treated with different enzymes. With each of the seven types of treatments the independent parameters studied were minimum and maximum enzyme dosage, and incubation times of 1 hour and 3 hours. The juices for each treatment were prepared in triplicate, and samples from treated juices were taken in triplicate.

From lingonberry juice samples, 34 volatile organic compounds were identified by comparing their mass spectra with those in the mass spectral library. The impact of different treatment conditions on these compounds was determined by using multivariate statistical methods. Peak areas were expressed with respect to internal standards. Incubation with enzymes increased the total concentrations of volatile organic compounds all around more than incubation without enzymes. Pectinases derived from a classic strain of *Aspergillus niger* increased the yields of juice and concentration of volatile organic compounds the most. The compounds identified were generally terpenes, alcohols, aldehydes, esters and ketones present in small quantities. VOCs with highest overall concentrations found were ethyl acetate, ethyl benzoate, 2-methyl-3-buten-2-ol, benzaldehyde, pentanal, hexanal, undecane, α-Pinene, linalool L and diacetyl.

KEYWORDS: Volatile organic compounds (VOCs), enzymes, lingonberry, flavour, SPME–GC-MS

Contents

FIGURES AND TABLES

Abbreviations

GC-MS	Gas chromatography–mass spectrometry
GC-O	Gas chromatography olfactometry
SPME	Solid-phase microextraction
PCA	Principal Component Analysis
RI	Retention index
VOCs	Volatile organic compounds

1 Introduction

An increasing demand for various berries for consumer and industrial needs has been identified in Europe. Although many berries are grown in Europe, demand is much higher than European production. This rising demand is in literature attributed to consumers' increased knowledge of the health benefits of berries and thus an interest in consumption of berry based products (CBI and Ministry of Foreign Affairs, 2018). Lingonberry (*Vaccinium vitis-idaea L*.) has received lesser attention than cranberry (*Vaccinium macrocarpon Ait*.) and bilberry (*Vaccinium myrtillus*), which has led to less increase in lingonberry consumption, when in comparison to cranberries or bilberries. These berries are often consumed as fresh fruits and processed products, such as dietary supplements and juices (Alejandro Vazquez-Cruz et al., 2012; Bujor et al., 2018). Sensory studies have demonstrated that for consumer acceptance and preference for berries the key properties are taste, aroma, and appearance. These properties are followed by potential health benefits gained from berry consumption (Miettinen, 2004; Verbeke, 2006). Verbeke (2006) concludes that it is a very risky strategic option to bet on consumer willingness to compromise on the taste, aroma, or appearance of foods for potential health benefits.

Many consumers base their perception of quality on sensory attributes, and the aroma of berries is one of the key properties determining the perception and acceptability of products by consumers. Aroma is a complex mixture of many volatile compounds. Berry fruits produce hundreds of volatile and non-volatile aroma compounds contributing to the overall flavour, and the flavour depends on several factors such as concentration and composition of these volatiles, climate and soil properties, growing practices, maturity stage and storage conditions. All plants can emit volatile organic compounds (VOCs), and genotypic variation can be seen in the content and composition of these molecules. Is essential to identify the key VOCs, that carry the unique aroma character of the berry, as they provide the principal characteristic flavour and sensory identity of the berry. As aroma is one of the most important traits of fruit quality, the study of VOCs has gained increasing attention in recent years (Hadi et al., 2013).

2 Berries

2.1 The characteristics of berries

The composition of berry fruits consists of flesh tissue, peel, and seeds or stones. Rigid cell wall holds together the flesh cells, which can be many micrometres thick. These cell walls gives the fruits a defined shape and protects them from external shocks and internal pressure. The major polysaccharides are pectin, hemicelluloses and cellulose in the primary cell wall (Whitehurst and Oort, 2010). Most commonly berries are consumed either as fresh, frozen, or dried. They are as well commonly used in the food industry, where they are processed into food products and dietary supplements. The most commonly consumed genera include: *Fragaria* (strawberries), *Rubus* (raspberries, black-berries), *Vaccinium* (blueberries, bilberries, lingonberries, cranberries), and *Ribes* (currants, gooseberries) (Alejandro Vazquez-Cruz et al., 2012).

2.2 Lingonberry

In Finland lingonberry (*Vaccinium vitis-idaea*), also known as cowberry, along with bilberry is the most abundant berry species found in the forests. Lingonberry plant is a wild shrub, whose fruits and aerial parts are rich in bioactive phenolic compounds. Lingonberry plants can be found in different parts of Europe, northern America and northern Asia (Ek et al., 2006; Tian et al., 2018). The lingonberry plant requires plenty of sun light and dry growing conditions. The plants can be found typically in light pine-dominated (sub-xeric) heath forests (figures 1 and 2).

Figure 1. Flowering lingonberries in a typical Finnish xeric heath forest photographed in Raisio, Finland 2.6.2018.

Figure 2. Ripening lingonberries in a xeric heath forest photographed in Kaarina, Finland 11.8.2018.

The flavour of lingonberries is described in literature as acidic, bitter and astringent. This is due the high amount of organic acids, especially citric acid. Lingonberries contain excessive amounts of benzoic acid (0.6–1.3 g/L free benzoic acid in lingonberry juice), and high amounts of other organic acids leading to a pH of below 4, which contributes to the acidity of the berry

(Viljakainen et al., 2002). Lingonberries are known to contain a relatively high concentration of sugars, but the sweetness is probably masked by acids, including benzoic acid (Viljanen et al., 2014). When lingonberry juice is stored in warm conditions, the concentration of benzoic acid in is known to increase even more. At low pH benzoic acid inhibits both the growth of bacteria and yeast. Thanks to these traits, benzoic acid is one of the most used preservatives in foods (Brul, 1999). As most microorganisms cannot ferment processed lingonberries because of the high benzoic acid concentration, lingonberry products can in most cases be conserved without addition of preservatives (Visti et al., 2003).

In Finland picking wild berries has maintained its popularity as a household and recreational activity. This popularity can be attributed to the Finnish “everyman’s rights”. These rights grant unrestricted access to both private and public land, in which people have the right to pick berries and mushrooms. In other Nordic countries, which also maintain these rights, the popularity of berry picking has been in decline (Turtiainen et al., 2011).

In Finland the total yearly lingonberry yields vary in a range from 129 to 386 million kg. The utilisation rates of lingonberries have been from approximately 8% to nearly 10%, varying on a yearly basis. In 2011–2013 an average of 20.4 million kilograms of lingonberries were harvested in Finland yearly. These numbers are rough estimates, and because they are not based on accurate data about the total berry yield, they area also mostly hypothetical (Turtiainen et al., 2011) Figure 3 describes the flow of Finnish lingonberries from forests to households during this time. For the Finnish berry industry, it is troublesome that a growing portion of Finnish lingonberries is sold straight from the harvesters to relatives and friends or straight to institutional kitchens and restaurants.

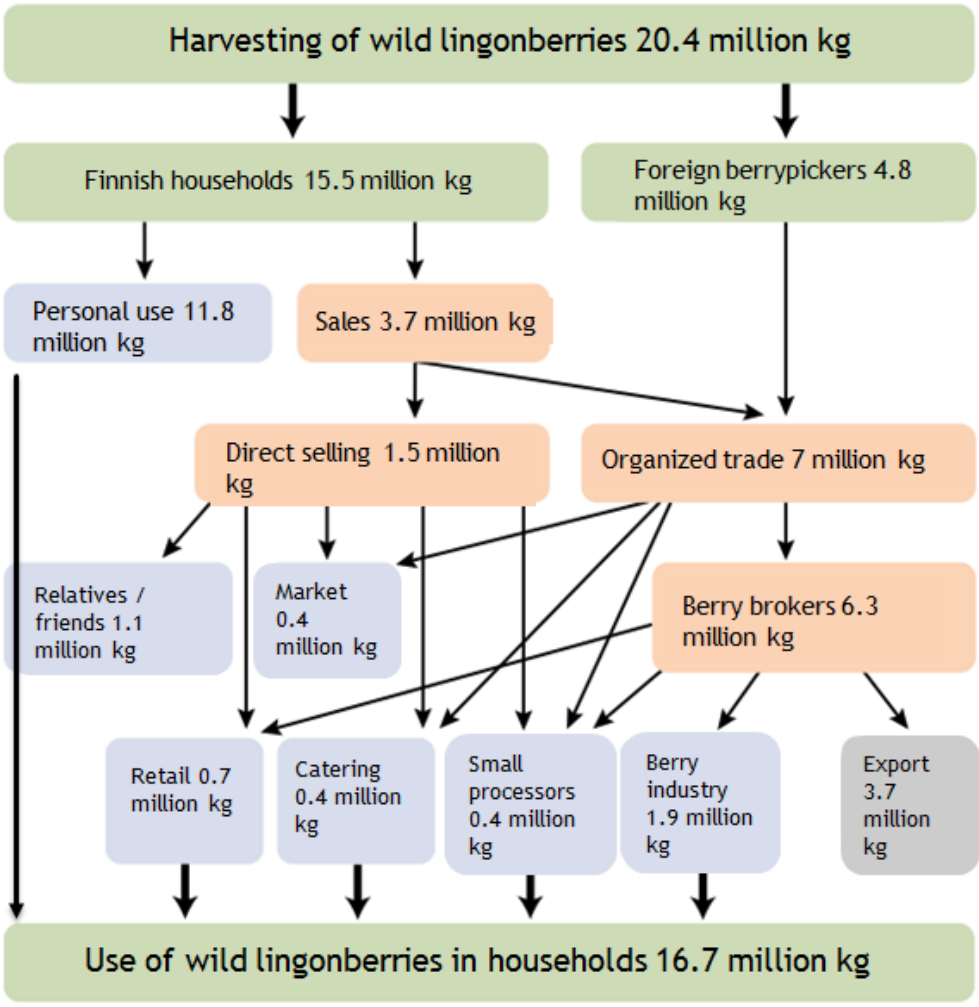

Figure 3. the flow of domestic lingonberries from forests to households in 2011-2013. Adapted from (Vaara, 2015).

As foreign demand for Finnish wild berries grows the domestic berry industry affords to use less domestic wild berries. This leads to importing of berries for the needs of domestic berry industry. The majority of Finnish wild berries are exported as unprocessed and thus the added value of processing remains untapped (Vaara, 2015).

3 Volatile organic compounds of fruits

3.1 Description of volatile organic compounds

By one definition volatile organic compounds (VOCs) are low-molecular-weight organic compounds, which evaporate easily at room temperature (Pennerman et al., 2016). The aroma of berries is resulted form the composition and concentration of VOCs in a genus- and species-specific way. Research on chemistry of fruit flavour focused earlier on the volatiles that characterize the specific fruit flavour. As strawberries and then grapes have had the greatest effort devoted to them, other berries have not been studied as vigorously. Currently most of the volatiles that characterize the flavour of berries with commercial importance have been identified (Reineccius, 2010). Chemically VOCs can be classified as aldehydes, alcohols, esters, ketones, lactones, and terpenoids (Hui, 2010).

The different concentrations of the VOCs often determine aroma properties. Many fruits produce VOCs in significant numbers as indicators of fruit ripening. They are produced in various concentrations: some in trace amounts, others in relatively large quantities. The concentrations found are not strictly correlated with importance to sensory properties. Some of those VOCs, which are below the thresholds of most analytical instruments, may still be detected by human olfaction. GC-MS is in terms of instrumentation the standard method in flavour studies. More than 360 VOCs have been identified in strawberries, over 270 in mangos, and over 147 in blackberries. These VOCs are formed and impacted by the studied species, cultivars, cultural managements, maturity and postharvest handling and storage (Hui, 2010; Reineccius, 2010). Only a few of the VOCs emitted by strawberries are the major ones contributing to the characteristic strawberry aroma. These contributing VOCs in strawberries are predominately esters, which comprise from 25% to 90% of the total VOCs in ripe, fresh fruit. In different strawberry cultivars furanones, ketones, sulphur and terpenes compounds are some important contributors to characteristic aroma. in strawberry fruit alcohol dehydrogenase and acyltransferase may be two enzymes involved with synthesis of ester VOCs. Mango fruits flavour is contributed mostly by monoterpenes, esters, and lactones (Hui, 2010; Kafkas and Kafkas, 2005; Schieberle and Hofmann, 1997).

All the VOCs do not give humans an odour sensation, especially in the low quantities they are often found in food matrices. Only some of the volatile compounds create and odour sensation when reaching the olfactory epithelium. In the olfactory epithelium they dissolve into the mucus, and may bond with olfactory receptors creating an odour sensation (Kalua et al., 2007). Flavour is given by perception of both taste and odour. The perception of both are also impacted by other sensory inputs. While most of the flavour compounds interact with the olfactory receptors in the nose, some of these flavour compounds also have an impact on the taste. Many natural VOCs have chiral centres and can exist as enantiomeric forms. These different enantiomeric forms can even have different aroma characteristics, and significantly different sensory thresholds (Malowicki et al., 2008).

3.2 Volatile flavour-active compounds in *Vaccinium* species

The aroma of berries of *Vaccinium* species is impacted by hundreds of volatile compounds, including acids, aldehydes, alcohols, esters, ketones, and terpenes. These volatile compounds are detectable in *Vaccinium* species with high variability according to environmental, genetic, and the stage of ripening differences (Farneti et al., 2017; Viljanen et al., 2014; Zhu et al., 2016).

3.2.1 Lingonberries

The volatile flavour-active composition of lingonberries (*Vaccinium vitis-idaea*) has only been studied previously in two studies. The first study conducted in the 1960's studied volatiles from extracted essential oil (Anjou and Sydow, 1967). The more recent one by Viljanen et al. (2014) made more throughout insights on bioprocessed lingonberries and identified 38 volatile chemical compounds (8 aldehydes, 6 ketones, 7 alcohols, 7 terpenes, 5 esters, 2 acids and 3 other compounds) by SPME-GC/MS. Aldehydes octanal and nonanal were related to lingonberry-like flavour in sensory evaluation. Of the acids 3-methylbutanoic acid was related to sweetness along with sugars glucose and fructose.

Figure 4. Structure of octanal.

Figure 5. Structure of nonanal.

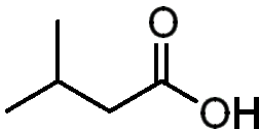

Figure 6. Structure of 3-methylbutanoic acid.

Anjou and Sydow (1967) describe 2-methylbutanoic acid as the most characterizing aromatic compound in lingonberry as the typical lingonberry-like aroma disappears when 2-methylbutanoic acid is neutralized.

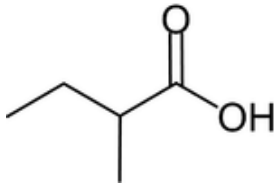

Figure 7. Structure of 2-methylbutanoic acid.

3.2.2 Blueberry

Farneti et al., (2017) characterized blueberry aroma from four different *Vaccinium* species: *V*. *corymbosum* L., *V. virgatum* Aiton, *V. myrtillus* L., and *V. cylindraceum* with 106 compounds. The compounds that are synthesized by the fruit in the ripe stage are mostly responsible for the recognisable blueberry aroma. These compounds include linalool and majority of monoterpenes, (Z)-2-hexen-1-ol, and hexanal. In the last stage of ripening are synthesized esters, such as ethyl acetate, methyl isovalerate, ethyl isovalerate, methyl 2-methylbutanoate.

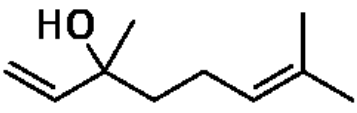

Figure 8. Structure of linalool L.

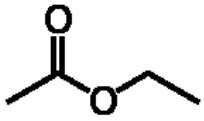

Figure 9. Structure of ethyl acetate.

3.2.3 Cranberry

The key aroma volatile compounds in cranberry (*Vaccinium macrocarpon* Ait.) according to (Zhu et al., 2016) are hexanal, hexen-1-ol, (E)-2-hexenal, pentanal, hexanol, β-ionone, linalool, 2-methylbutanoic acid, benzoic acid, cis-3-hexenyl acetate, and 4-mercapto-4-methyl-2-pentanone. These aldehyde, terpenoid and sulfuric compounds were found to contribute the most to the aroma profile of the studied cranberries.

Figure 10. Structure of hexanal.

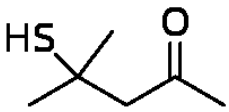

Figure 11. Structure of 4-Mercapto-4-methyl-2-pentanone.

Cysteine, cystine, and methionine are sulfur-containing amino acids, which are the major precursors of the sulfur-containing compounds (Zhu et al., 2016).

3.3 Factors influencing volatile organic compound composition

As various factors influence the composition of VOCs produced in fruits, it is important understand these factors in order to improve the organoleptic quality of fruits. The volatile composition in fresh fruit is continuously changing due to the complex nature of the volatile profiles. The VOCs that are mainly responsible for fruit flavour are biosynthesized through metabolic pathways. This biosynthesis occurs during maturing, postharvest handling, and storage. For most fruits the production of VOCs is mostly related to fruit ripening (Hui, 2010).

The genetic background of fruit cultivars impacts the composition and concentration of VOCs clearly. With tomatoes the insertion of *rin* gene, which reduces ethylene production, results in some reduction in flavour volatiles and flavour quality. Cultivated strawberries are rich with monoterpene-linalool and the sesquiterpene nerolidol, while wild-type varieties are rich in oleafinic monoterpenes and myrenyl acetate (Hadi et al., 2013; Nile and Park, 2014).

Maturity of the fruit is considered the critical factor impacting the quantity and quality of VOCs in fruit. Fruit should be harvested at the right maturity to ensure best possible VOC quality. The realities of economics force fruit producers to harvest immature fruits in order to increase storage and shelf life, resulting in lacking flavour due to incomplete biosynthesis of VOCs during maturing. In strawberries furanone and esters are present in fully matured fruit, as in immature fruits C_6 aldehydes are dominant. In cantaloupe melons total VOCs increase linearly with increasing maturity. During maturing factors such as sunlight, fertilization, chemical applications, and water availability have an impact on flavour. The highest quantities of VOCs in grapes can be achieved when the vines are under moderate nitrogen supply and mild water deficit. The use of Aminoethoxyvinylglycine (AVG) on apples and pears, which is a plant regulator used delaying fruit maturity, leading to benefits such as a reduction in pre-harvest fruit drop and improved fruit quality, is known to adversely affect the production of some VOCs in “Golden Delicious” apples (Hadi et al., 2013; Salas et al., 2011).

During storage temperature has a significant impact on the flavour of fruits. In honeydew melons and cantaloupe storage temperature of 4 °C caused the decline of acetate esters and increase of non-acetate esters after two days of cold storage. This change is suspected to lead in to an imbalance of characteristic VOCs to these fruits (Beaulieu, 2007). Storage atmosphere is modified in order to maintain the quality of fruits for extended periods. Controlled atmosphere conditions may alter the production of VOCs by reducing the fruits capacity to produce ethylene. (Hadi et al., 2013).

An organoleptic assay should support the chemical analysis of VOCs, since all the volatile compounds are not responsible for the aroma. An organoleptic assay is used to identify the "character impact" and the "contributory" flavour compounds to create a description of the odour of the components (Rizzolo et al., 1992).

VOCs can be classified in two groups: as primary or secondary. Primary VOCs are present in intact fruit tissue, and secondary VOCs are produced because of tissue disruption. Often VOCs are only released when tissue disruption occurs. The disruption causes previously compartmentalized enzymes and substrates to

interact. This disruption can be for example mashing of berries mechanically. Some VOCs can found as bound to sugars as glucosinolates or glycosides. Glycosides of VOCs in berries are mainly *O*-diglycosides, *O*-*β*-D-glucosides, and in lesser amounts, triglycosides. As glycosidically bound volatiles are often found in greater proportion than free volatiles, they are important source of VOCs. The fruits maturation, processing and storage, or the action of enzymes, acids or heat may release the odorous aglycones from the sugar moiety. It should be taken in to consideration when performing analysis of VOCs, that the aroma profiles and final aroma interpretation will differ when taking samples from either intact or disrupted fruit tissues (Hui, 2010).

4 Enzymes

“Catalysis can be described formally in terms of a stabilization of the transition state through tight binding to the catalyst.”

-William P. Jencks, article in Advances in Enzymology, 1975

4.1 Enzymes generally

In 1878 the term “enzyme” was first coined by Kuhne, and in 1883 Duclaux coined the term “substrate”. Although enzyme activity had been identified previously, it was not until 1926 that Sumner crystallized the first enzyme (urease) from bean flour at Cornell University, demonstrating that enzymes existed (Campbell and Drake, 2013).

The application of enzymes in the food processing is an important branch of biotechnology. Enzymes are the proteins that catalyse virtually all the chemical reactions occurring in biological systems, and thousands of enzymes have been identified and characterized. Enzymes cause enormous increases in reaction rates by lowering reactions energy barriers through optimal orientation of the reactants often using temporary bond formation between substrate and enzyme. All the processes in nature require enzymes in order to occur at significant rates. When a proteins native structure is altered its new structure, or conformation, is called denatured. The potential for inactivation of enzymes by pH change, temperature increase, UV-bleaching, etc. follows the same denaturation principles as proteins normally. Enzymes are selective for their substrates and therefore catalyse only a few reactions form among many possibilities (Campbell-Platt and International Union of Food Science and Technology, 2009; Whitehurst and Oort, 2010).

Enzymes are globular proteins ranging in size from just over 60 to more than 2500 amino acids. The three-dimensional structure of the enzyme determine its activities. It is noteworthy that only a small part of the enzyme molecule is directly involved in catalysis, as most enzymes are much larger than the substrates they act on. The small section involved in the catalysis contains not more than a few amino acids and is called the active site of the enzyme. Normally the substrate is bound by the enzyme near, or even in, the active site. There are several factors

influencing the reaction rate at which enzymes proceed. These include enzyme concentration, substrate concentration, temperature, pH, and the presence of any activators or inhibitors (Whitehurst and Oort, 2010).

4.2 Enzymes in food processing

Enzymes have several advantages in food processing and production. Enzymes allow milder processing conditions, resulting in preservation of valuable attributes of food and food components, as they can catalyse reactions under very mild conditions. They also can improve and modify the nutritional, functional and sensory properties of ingredients and products. As enzymes are more specific in their action than chemical reactants, higher quality products can be generated via enzyme-catalysed processes. These enzyme-catalysed processes will have fewer side reactions and by-products generating higher quality products. The use of enzymes in the extraction of biomolecules from plants is a promising alternative to traditional solvent extraction methods. Enzymatic extraction as a technology is gaining more interest for being eco-friendly, benign, sustainable and efficient (Nadar et al., 2018; Whitehurst and Oort, 2010).

Some of importance to the food industry include the production of high fructose corn syrups by using glucose isomerase, saccharification of starch by amylases in baking and brewing, juice clarification by using cellulases and pectinases, production of low lactose milk by using lactase, cheese making by rennin, and meat tenderization by proteases such as papain, bromelain and ficin. The cell wall degrading enzymes (hemicellulase and cellulose) are used to extract vegetable oil (olive and canola / rape seed) in aqueous process by liquefying the structural cell wall components of the oil-containing crop. Enzymatic treatment is used to extract a higher yield in juice processing. The enzymes used are mainly pectinase, cellulose, and hemicellulase. Enzyme addition increases the release of various phenolics and increases juice extraction yield. Enzymatic treatment also improves juice appearance and quality in terms of reduced viscosity and improved filterability (Campbell-Platt and International Union of Food Science and Technology, 2009; Whitehurst and Oort, 2010).

Regarding the VOCs enzymes can have important applications for the industry. Naturally non-odorous and non-volatile precursors represent an important source of fragrant compounds in aroma. During fruit maturation *β*-glucosidases, which are endogenous enzymes, release this aroma potential. The extraction efficiency of traditional extraction techniques is decreased by the existence of various polysaccharides such as hemicelluloses, starch, and pectin in large amounts inside the cell wall described in figure 8 (Rocha et al., 2005).

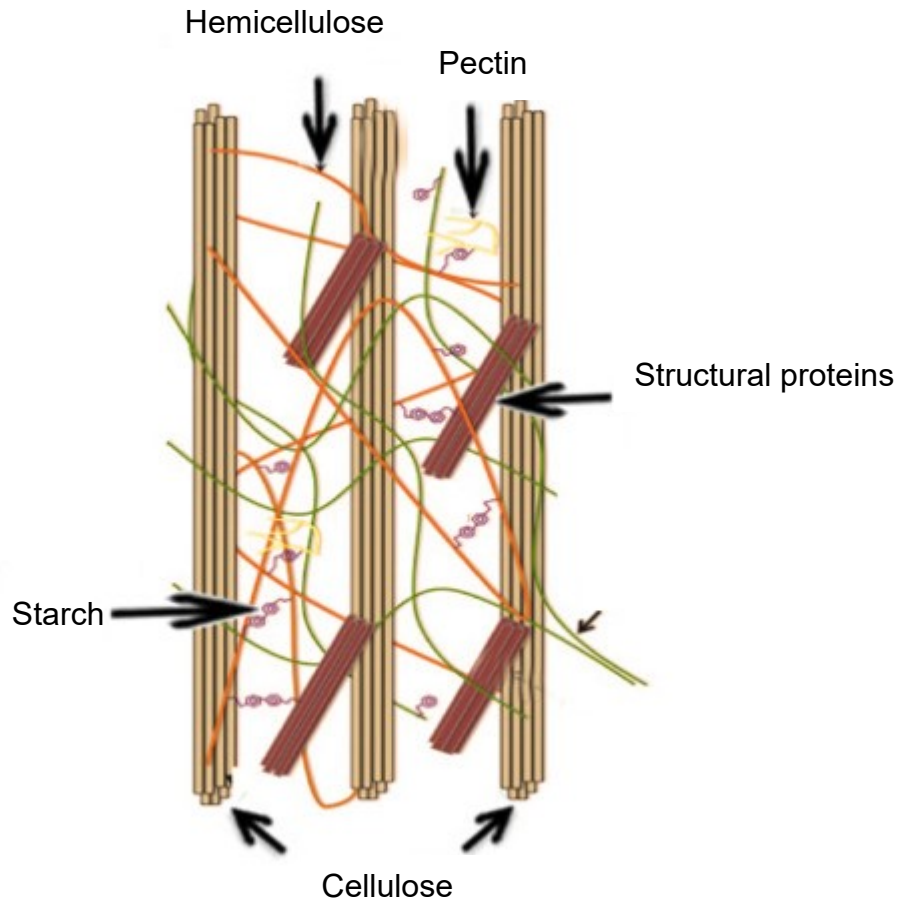

Figure 12. Representation of primary cell wall structure of plant material. Adapted from (Acosta-Estrada et al., 2014).

Cellulose, hemicellulose and pectin can be hydrolysed by using *β*-glucosidase, cellulose, and pectinase enzymes. At the molecular level cellulose is an unbranched polymer consisting of 1000 to 1 million *D*-glucose units, linked together with *β*-1,4 glucosidic bonds. *β*-glucosidase can break these *β*-1,4 glucosidic bonds in glucosides. The naturally occurring endogenous *β*-

glucosidase enzymes are low in activity and cannot release the whole aroma potential. Therefore experiments have been performed with exogenous *β*-glucosidases. (Nadar et al., 2018). The use of these exogenous *β*-glucosidases allow the release of volatile compounds in higher concentrations, producing juices with richer aroma, being more intense and complex (Rocha et al., 2005). Pectic substances are chemically high-molecular-weight carbohydrate polymers which are present in the cell wall of higher plants. Pectins consist of a chain of galacturonic acid units which are linked by *α* -1,4 glycosidic bonds (Flutto, 2003). Pectinase enzyme splits polygalacturonic acid into monogalacturonic acid by opening glycosidic linkages (Mojsov, 2016). Cellulases are the enzymes that hydrolyze *β*-1,4 linkages of insoluble cellulose at the solid/liquid interface, generating soluble oligosaccharides, cellobiose, and glucose (Mojsov, 2016).

Enzymatic degradation and extraction of the biomaterial depends upon the type of enzyme and their characteristic property to carry forward reactions with accurate specificity, incubation time, incubation temperature, enzyme concentration, agitation, pH, use of different enzyme combinations and their ability to react under mild conditions. For every enzyme the optimum pH for enzymatic hydrolysis is different. Very high or low pH values usually result in a total loss of activity for many enzymes. pH is also a factor in the stability of enzymes (Nadar et al., 2018; Whitehurst and Oort, 2010). The optimal temperature is also dependant on the specific enzymes used. Like most chemical reactions, the rate of an enzyme-catalysed reaction increases as the temperature is raised. The activity of most enzymes will increase by 50 to 100% by a ten degree rise in temperature. Variations in reaction temperature as small as 1 or 2 Celsius degrees may introduce changes of 10 to 20% in the results (Worthington Biochemical Corporation, 1972). Many enzymes are adversely affected by high temperatures, which complicates things when searching the optimal treatment temperatures. Incubation in too high temperatures can cause a loss in enzyme activity alongside with the inactivation of proteins and other bioactive molecules. Incubating at too low temperatures does not accelerate the action of enzymes consequently leading to less extraction efficiency of various biomolecules (Nadar et al., 2018). Prolonged incubation time may increase the solubilisation of plant cell wall components, but too long incubation time can lead to lesser energy

efficiency and product quality (Babbar et al., 2016). Higher enzyme concentration promote plant cell wall solubilisation, which may be a result from better interaction between the enzyme and substrate (Zhang et al., 2007).

4.3 Application of exogenous enzymes and their impact on food volatile aroma compounds

The most commonly used exogenous enzyme applications in food matrices and studies presenting the impact of exogenous enzymatic treatments in food matrices are described below in some detail.

4.3.1 Wines and fruit juices

The studies that are presented next suggest that wine aroma can be improved by hydrolysing odourless aroma precursors into VOCs. Hydrolysis can be enhanced by using exogenous enzymes such as glycosidases, *β*-glucosidases and pectinases. Higher polysaccharide content might cause higher retention of volatile compounds.

Glucosidases are used to improve the flavour of wines by releasing glycosidically bound flavour precursors and especially volatile terpenes. This is because in grapes terpenols are mostly found in odourless, glycosidically bound forms. It has been shown that the inclusion of exogenous enzymes during or after fermentation is the most effective way to improve the hydrolysis of aroma precursors and to achieve an increase in wine aroma (Aryan et al., 1987; Longo and Sanromán, 2006). The study of the aromatic potential of apricot juice and muscat wine revealed in the 1990's that along with a free fraction of volatile terpenols there exist naturally non-volatile and non-odorous precursors and they represent a significant source of VOCs. During fruit maturation this aromatic potential was naturally revealed by endogenous *β*-glucosidases. But since these endogenous enzymes cannot liberate the whole aromatic potential because of their low activity, hydrolytic experiments were made with exogeneous *β*-glucosidases. Immobilized and free *β*-glucosidases were used to treat the apricot fruit juice and muscat wine. These enzymes were more efficient in releasing bound terpenes without changing the aromatic character of the terpenes than acid hydrolysis. A following GC−MS analysis indicated a significant increase in the concentrations of VOCs. In the apricot fruit juice namely linalool, *α*- and *γ*-terpinene, *α*-terpineol,

2-phenylethanol, and *α*-pinene concentrations increased. In the muscat wine the concentrations of nerol, geraniol, linalool, 2-phenylethanol, and benzyl alcohol increased (Gueguen et al., 1996).

Enzyme preparations from *Trichoderma harzianum* and *Aspergillus niger* were used for aroma release (Sun et al., 2018). Studies have shown that these pectinase enzymes can impact the wine aroma by increasing VOC concentrations. In a study in winemaking the use of a pectinase enzyme preparation containing relevant glycosidase activities resulted in increase in the levels monoterpenes, C13-norisoprenoids, and benzene derivatives. These increases may have resulted from the hydrolysis of glycosidically bound aroma compounds (Cabaroglu et al., 2003). The addition of exogenous glycosidases enhances greatly aromas in wines in relation with the aromatic potential of grape varieties by hydrolysing odourless aroma precursors into volatile varietal compounds, such as terpenes and C13-norisoprenoids. Sensory evaluations confirm, that the improvement is obvious for red and for white wines. In these sensory evaluations enzymatically treated wines were always evaluated fruitier and more intense (Grassin and Fauquembergue, 1996). However, Sun et al. report that some glycosidase preparations of fungal origin can cause collateral hydrolysis reactions and form unwelcome aromas (Sun et al., 2018). Leino and Kallio, (1993) fermented black currant juice in to wine using yeast enzymes. They discovered the concentrations of volatiles such as ethyl hexanoate, ethyl octanoate, and some esters to increase considerably. They also observed terpenoids and some esters to decrease in concentration and attributed this to the fermentation process.

In a study pectolytic enzymes were used in apricot, peach and pear fruit juice manufacture. These enzymes affected the behaviour of the volatile compounds by modifying the polysaccharides composition. Volatile compounds with highest concentrations determined in untreated apricot juices were benzaldehyde, some esters, norisoprenoids, and terpenoids. The most abundant volatile compounds in untreated peach samples were benzaldehyde, methyl and ethyl acetate, and some lactones. The most abundant volatile compounds in untreated pear samples were hexanal, cinnamaldehyde, methyl and ethyl decadienoates, and farnesenes. Farnesene is a constituent of the natural coating of apples and pears

and other fruit. The used enzymes enhanced the flavour of apricots in pleasant aroma volatiles such as terpenes and norisoprenoids. Peaches treated with pectolytic enzyme contained the lowest concentrations of VOCs. The authors constitute this to the higher retention of volatile compounds caused by high polysaccharide content in these samples. In pear fruit the pectolytic treatment did not increase the concentration of the most important VOCs of pears, decadienoate esters (Riu-Aumatell et al., 2005).

Terpenes might not be released from their glucoside precursors in blackcurrant nectar by the action of *β*-glucosidase enzyme treatment. In a study on black currant nectar only *α*-humulene changed significantly in concentration, and it decreased. The study proposed that in this certain matrix the *β*-glucosidase activity was inhibited by the glucose and fructose from the black currants (Iversen et al., 1998).

4.3.2 Tea

Recent advances showcase the untapped potential in tea industry for exogenous enzymatic treatments in strengthening the aroma volatile compositions. Monoterpene alcohols and aromatic alcohols are present as monosaccharide or disaccharide glycoside precursors in fresh leaves of tea plants. It has been reported that the quality of tea has been improved by introducing exogenous enzymes such as polyphenol oxidase, peroxidase, tannase, cellulase, pectinase, protease, laccase, *α*-galactosidase, and *β*-glucosidase into tea processing in order to hydrolyse glycosidic aroma precursors (Ni et al., 2017; Su et al., 2010; Zhang and Du, 2015). Ni et al. (2017) showcase these advances in a recent study, where green tea was treated with enzyme extracts of *Aspergillus niger.* The treatment had a significant impact on sensory indexes, increasing toasty and mushroom aroma, and volatile constituents. The contents of eucalyptol, hexanol, benzaldehyde, cis-3-hexenol, and 1-octen-3-ol increased. Furthermore, GC–O analysis showed that an increase in 1-octen-3-ol strengthened the mushroom aroma.

4.3.3 Applications in bakery, meat, and dairy products

In bread making industrial enzyme preparations often have a role as a secondary effect in flavour formation. Fermentation and baking are most of the time the primary sources of the flavour of bread. The three enzymic systems that are related to bread flavour are proteases, amylases, and lipoxygenases. The formation of flavouring peptides is the only direct role of enzymes in bread flavour. The main role of enzymes regarding VOCs is to form precursors related to flavour-forming processes. As such enzymes are of foremost importance in generating bread flavour (Martínez-Anaya, 1996).

In meat industry exogenous enzymes are used to tenderize meat through proteolysis. Consumers' perception of overall meat quality is impacted by multiple properties, but tenderness is viewed as one of the most important (Sullivan and Calkins, 2010). The flavour of processed meat is a result from enzymatic action (Toldrá and Flores, 2000). Aroma is considered the most important quality parameter of ham. The ham aroma is produced by chemical and enzymatic mechanisms during the post-mortem process via proteolysis and lipolysis, which are the main enzymatic reactions impacting formation of flavour precursors and meat flavour (Maehashi et al., 1999; Marušić et al., 2011). During ham production the formation of numerous volatile compounds is resulted from lipid disintegration and subsequent oxidation of free fatty acids. These VOCs include aldehydes, alcohols, esters, aliphatic and aromatic carbohydrates, short-chain fatty acids, and furan derivatives (Marušić et al., 2011). Bitter peptides resulted from the hydrolysis of proteins however often limit the use of protein hydrolysates in food products (Longo and Sanromán, 2006).

The use of lipases and proteases may result in increased concentration of VOCs in meat products. A study investigating protease treatment of crayfish-processing by-products identified enzymatic treatment causing an increase in the concentration of pyrazines and benzaldehyde (Baek and Cadwallader, 1996). In another study protease-mediated catalysis was found as an alternative to produce a savoury flavouring product, which is traditionally produced by heating a protein source at acidic pH, hydrolysed vegetable protein. In this study enzymatic treatment increased alcohols formation, and pyrazine formation through Maillard reactions (Aaslyng et al., 1998).

Milk contains several endogenous enzymes which may affect flavour, such as proteinases, lipases, lactoperoxidase, and catalase. Exogenous enzymes are often added to milk or milk products for functions such as flavour (lipases in certain cheeses), milk clotting (chymosin), catalase to deactivate hydrogen peroxide), and bleaching of whey products (fungal peroxidases). Enzymes are also produced during the cheesemaking process from nonstarter bacteria or starter culture (Campbell and Drake, 2013). In a dairy related study, the use of enzyme preparations including lipases, esterases, proteases, and peptidases to produce enzyme-modified flavour-enhanced cheese was researched. When incubating curd with enzymes up to 30-fold flavour enhancements were reached when compared to non-incubated natural cheese (Kilcawley et al., 1998).

In another study the effect of four different commercial lipases on the flavour profiles of lipolysed milk fat was researched. The most potent volatiles resulting from lipolysis were determined with gas GC–MS and GC–O. Forty-six volatile compounds were identified and quantified by GC–MS. Nineteen volatile compounds were major contributors to the characteristic flavour of the lipolysed samples. The most contributing aroma compounds resulting from the use of these lipases were hexanoic acid, 2-nonanone, 2-undecanone, butanoic acid, δ-dodecalactone, and hexanal (Wang and Xu, 2009).

5 Aim

In this master's thesis work the VOCs in lingonberry juice were characterised and the impact of different enzymatic treatments (pectinases, cellulase, *β*-glucosidase) on these identified VOCs were studied. The enzymatic treatment parameters studied were incubation time and enzyme concentration with each enzyme used. The impacts of enzymatic treatments on VOCs were studied with GC-MS. The volatile composition of lingonberries has only been studied previously by Anjou and Sydow (1967) and impacts of enzymatic treatment by Viljanen et al. (2014).

Aldehydes octanal and nonanal were expected to be found, as they were related to lingonberry-like flavour in sensory evaluation in a study by Viljakainen et al. (2014). 2-methylbutanoic acid was expected to be found in large quantities as Anjou and Sydow (1967) propose.

In addition to the free fraction of volatile compounds, the precursors which are naturally non-odorous and non-volatile, also represent an important source of fragrant compounds in aroma. As presented in the literature overview, it would be expected that the VOC composition would be modified by hydrolysing odourless aroma precursors into VOCs. It was expected that some or all the enzymatic treatments would increase the measured quantities of VOCs in the samples. It was also expected that higher enzyme concentration and incubation time would result in better interaction between the enzyme and substrate, thus promoting plant cell wall solubilisation and release of VOCs.

6 Materials and Methods

6.1 Preparation of lingonberry juices

Seven types of lingonberry juice samples were studied: enzymatically treated with Rohapect Classic (AB Enzymes GmbH, Germany), Rohament CL (AB Enzymes GmbH, Germany), Rohapect UF (AB Enzymes GmbH, Germany), Beta Glucosidase 16L (Biocatalysts Limited, United Kingdom), NF10 (a research enzyme) and Without Enzyme & Heat treatment and Without enzyme. The independent parameters studied were incubation time and enzyme dosage.

A total number of 201 samples were studied with GC/MS. Figures 13 and 14 describe the flow of work. Before analysis the enzymes were inactivated with NaCl and an internal standard was added. Each sample was analysed in triplicate, and peak areas were expressed with respect to internal standard.

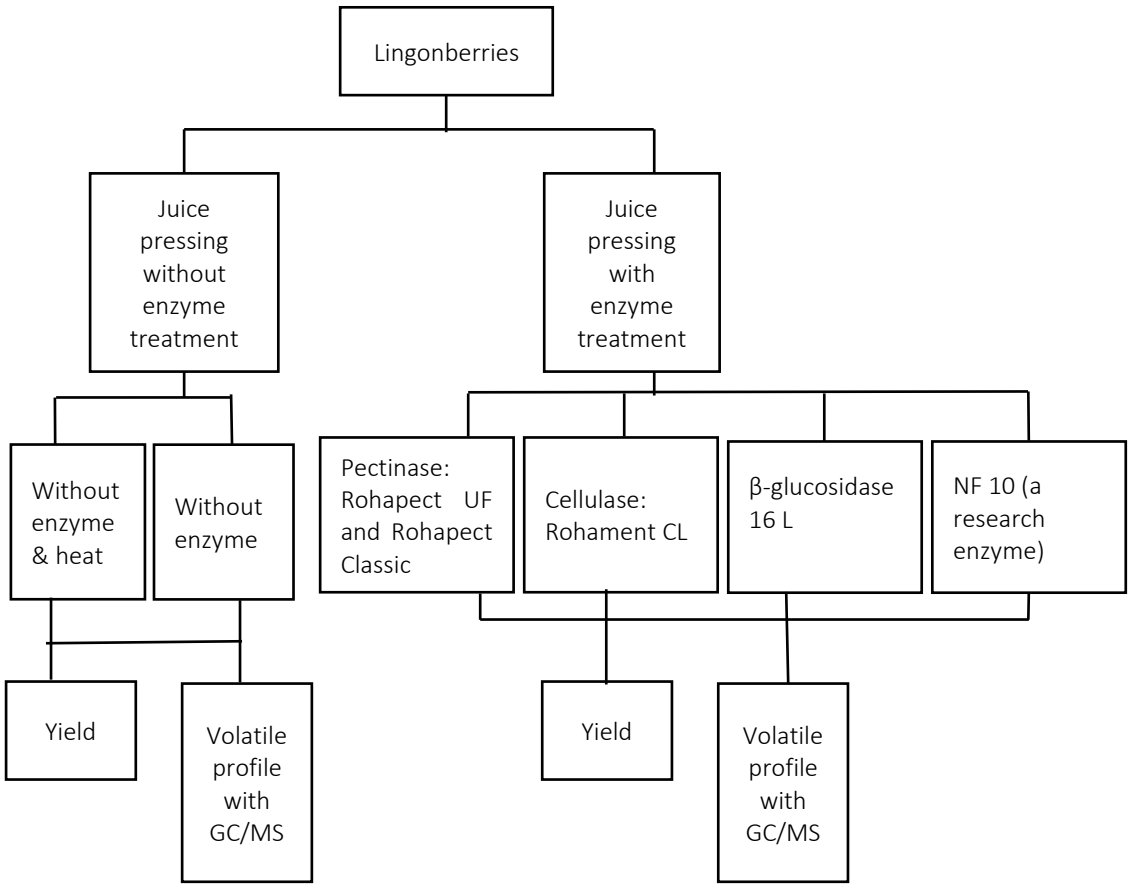

Figure 13. Lingonberry juice treatment and analysis chart.

Figure 14. Sample preparation scheme.

Each of the treatments were done in triplicate, and triplicate samples for the GC-MS were prepared from each of the treatments. In figure 14 the number 1 describes the one of the five enzymatic treatments (5*36=180 samples). Number 2 describes the "without enzyme" treatment (=18 samples), and number 3 describes the "without enzyme & heat" treatment (=3 samples).

6.2 Sample materials

Frozen, ripe lingonberries (*Vaccinium vitis-idaea*) of Finnish origin were obtained as frozen from a local distributor, Arctic International Oy c/o Marjex-tuotteet. (Nurmijärvi, Finland). The lingonberries were stored at −20 °C until use.

6.3 Enzymatic treatments and juice processing

Seven types of lingonberry juice samples were studied, of which two were references without enzyme treatment: (1) Without Enzyme & Heat treatment, (2) Without enzyme; (3) enzymatically treated with Rohapect Classic, (4) Rohament CL, (5) Rohapect UF, (6) Beta Glucosidase 16L, and (7) NF10.

Rohapect UF (CAS-No.: 9032-75-1) contains an enzyme complex, whose single enzyme activities are special pectinases and arabinases. The pectinase is derived from a “classic” strain of *Aspergillus niger*. Cellulase Rohament CL (CAS-No.: 9012-54-8) is an enzyme preparation for hydrolysing non-starch polysaccharides, containing cellulase for degradation of cellulose and other enzymes to degrade plant cell wall polysaccharides such as beta-glucans and xylans. Produced by controlled fermentation of a classical strain of *Trichoderma reseei*. Rohapect Classic (CAS-No.: 9033-35-6) is an enzyme preparation containing pectolytic activities produced by controlled fermentation of a classical strain of *Aspergillus niger*. Beta Glucosidase™ 16L (G016L) is an exo-carbohydrase containing cellulase and hemicellulase activities for removing the glucose entity and releasing the active flavour from inactive glucoside forms produced from *Trichoderma longibrachiatum*. NF 10 a developmental enzyme, pectinase with strong side activities, e.g. betaglucosidase

Figure 15. Used commercial enzymes.

Lingonberry juices were prepared in batches of 200 grams. Berries were thawed in a microwave oven, mashed with a Bamix mixer (Bamix M13, Mettlen, Switzerland) and warmed up in a water bath to the incubation temperature. Procedures and analyses were carried out by using different incubation times, dosages and various enzymes shown in table 1.

Table 1. Sample preparation scheme.

	Sample 1	Sample 2	Sample 3	Sample 4
Without Enzyme & Heat	X1=none X2=none			
Without Enzyme	X1=none X2=1h	X1=none X2=3h		
Rohament Classic	X1=minimum X2=1h	X1=minimum X2=3h	X1=maximum X2=1h	X1=maximum X2=3h
Rohament CL	X1=minimum X2=1h	X1=minimum X2=3h	X1=maximum X2=1h	X1=maximum X2=3h
Rohapect UF	X1=minimum X2=1h	X1=minimum X2=3h	X1=maximum X2=1h	X1=maximum X2=3h
Beta Glucosidase	X1=minimum X2=1h	X1=minimum X2=3h	X1=maximum X2=1h	X1=maximum X2=3h
NF10	X1=minimum X2=1h	X1=minimum X2=3h	X1=maximum X2=1h	X1=maximum X2=3h

All samples were made in triplicate. The independent parameters studied were enzyme dosage *X*1 (minimum / maximum) and incubation time *X*2 (1h / 3h). Incubation temperature was 50 °C for all samples except reference samples (“Without enzyme & heat”).

The enzymatic treatments were performed by incubating the mashed berry samples in a thermostatically controlled water bath under constant stirring in plastic Minigrip® reclosable bags. Juicing the enzymatically treated berry mashes was performed with HAFICO HP 2 H Tincture Press (FISCHER Maschinenfabrik, Germany) at 150 kg/cm^2 pressure.

6.4 Analysis of volatile compounds with GC/MS

For analysis of volatile compounds, 2 ml of lingonberry juice samples were weighed into 20-mL headspace vials. 4-methyl-2-pentanol was used as internal

standard (10 µg/sample). Samples were analysed by GC/MS according to a method optimized by Ph. D Alexis Marsol. The HS-SPME technique itself was invented in paper by (Arthur and Pawliszyn, 1990), and the method used in this work was a modification from the method used in (Marsol-Vall et al., 2018). The analytes were injected at 200 C in the split injector (split flow 10.0 mL/min) to the gas chromatograph (Thermo Scientific™ TRACE™ 1310, Switzerland) coupled with an MS detector (Thermo Scientific™, TSQ™ 8000 Evo Triple Quadrupole GC-MS/MS, Switzerland) and SPME autosampler (Thermo Scientific™, TriPlus RSH™ Autosampler, Switzerland). Analytes were separated on an SPB®-624 Capillary GC Column (60 m × 0.25 mm, df 1.40 µm; Sigma-Aldrich, Germany), with a constant flow of 1.6 mL/min, using helium as carrier gas. The temperature programme started at 50 °C with 3 minutes holding time, then increased 5 °C/min up to 160 °C, followed by 10 °C/min increase up to 225 °C, where the temperature was kept for 15 minutes.

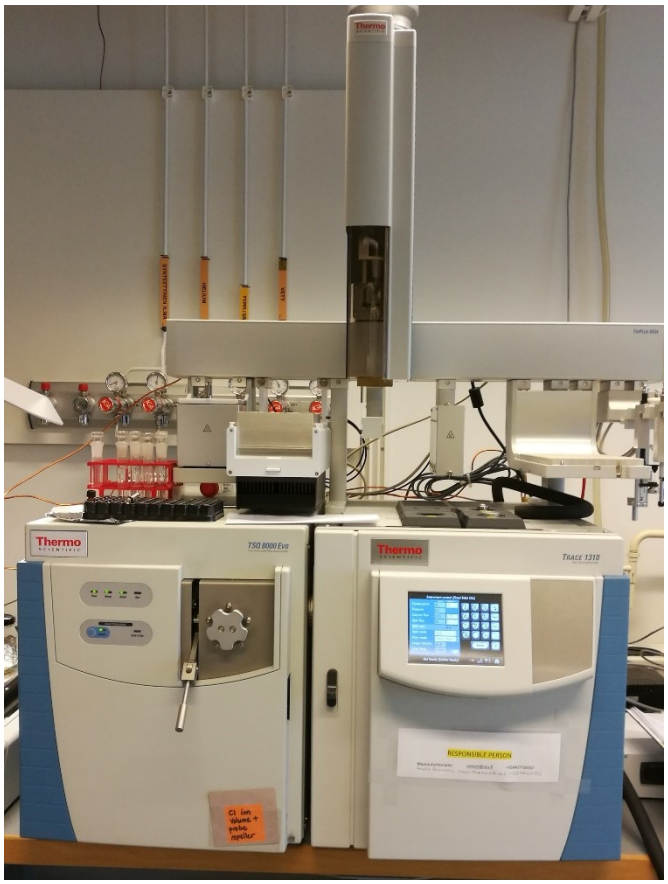

Figure 16. Trace 1310 gas chromatograph coupled to a TSQ 8000 EVO mass spectrometer and a TriPlus RSH multipurpose autosampler used in this work.

6.5 Data analysis and identification of volatile compounds

Compounds were tentatively identified by comparing their mass spectra with those in the NIST MS Search 2.0 Mass Spectral Library (National Institute of Standards and Technology, USA) and comparing with Kovats indices found in the literature. The literature sources compared with were Flavornet (Acree and Arn, 2004) and Viljanen et al. (2014). The Kovats index of each compound was calculated based on n-alkanes (C3–C26).

The formula to obtain Kovats indexes was

$$I = 100n + 100z\frac{(\log t\ RA - \log t\ Rn)}{(\log t\ RN - \log t\ Rn)}$$

where I is the Kovats index, n is the number of carbon atoms in the smaller n-alkane, z is the difference in the carbon atoms in the smaller and larger n-alkanes, A is the unknown compound, N is the number of carbon atoms in the larger n-alkane, and tR is the retention time (Nič et al., 2009).

The data was screened using TraceFinder™ 4.1 software to automatically integrate the chromatography peaks. Each sample was analysed three times and normalised peak areas were expressed with respect to internal standard (compound area/ISTD area). Principal component analysis (PCA) was applied to study the differences between different enzymatic treatments.

7 Results and discussion

7.1 Volatile compounds in studied lingonberry juices

A total number of 34 volatile chemical compounds were identified by GC-MS from lingonberry juice samples (Table 3); 8 aldehydes, 5 ketones, 2 alcohols, 8 terpenes, 6 esters, and 5 other compounds. In the literature, only two research papers were found on volatile compounds in lingonberries (Anjou & Von Sydow, 1967), in which essential oil was extracted from berries, and (Viljanen et al., 2014), in which only one enzymatic treatment with an enzymatic mixture was studied as a part of a larger bioprocessing study. A chromatogram of untreated lingonberry juice is presented in Fig 17.

When comparing the results from this study to those form Viljanen et al. (2014), some quick conclusions can be made. Firstly, the number of compounds identified is somewhat different, as described in table 2.

Table 2. Comparison of identified compounds between Nuutinen and Viljanen et al. (2014).

Group	Nuutinen	Viljanen et al. (2014)
Aldehydes	8	8
Ketones	5	6
Alcohols	2	7
Terpenes	8	7
Esters	6	5
Acids	0	2
Other	5	3
Total	34	38

The reason for this disparity is explained by differences in used GC-MS methodology, credibility of identification, and differences in used lingonberry material. Viljanen et al. (2014) describe aldehydes nonanal and octanal as having

a lingonberry-like aroma in sensory evaluations. Unfortunately, of those two only octanal could be identified reliably in this study. Of the acids Viljanen et al. (2014) highlights 3-metylbuthanoid acid as prominent one, being related to sweetness. This acid was also identified in this study but could not be taken in to account due to poor separation in most samples. Of the 34 identified compounds in this study, 15 are different from those identified by Viljanen et al. (2014). See table 3 for the identified compounds and a comparison of which compounds were found in both studies. The odour descriptions in table 3 are adapted from Flavornet (Acree and Arn, 2004), and are used when discussing the possible impact of the enzymatic treatments.

The concentration of volatile compounds changed during the enzymatic treatments and these changes are presented here on a treatment basis, taking in to account the independent parameters; incubation time and dosage.

Table 3. The identification of volatile compounds with their odour descriptions.

RI [C]	RETENTION TIME/MIN (PEAK NUMBER)	COMPOUND	FOUND IN VILJANEN ET AL. (2014) [B]	ODOUR DESCRIPTION [A]
	Aldehydes			
738	15.30 (10)	Pentanal	X	Almond, malt, pungent
843	19.53 (13)	Hexanal	X	Grass, tallow, fat
947	23.58 (15)	Heptanal		Strong fruity
1019	26.26 (18)	(Z)-2-Heptenal	X	Fatty, oily, with fruity overtones
1036	26.86 (20)	Benzaldehyde	X	Almond, burnt sugar
1047	27.25 (21)	Octanal	X	Fat, soap, lemon, green
1086	28.64 (26)	Undecane		Alkane
1199	32.36 (35)	Decanal		soap, orange peel, tallow
	Ketones			
-	8.12 (2)	Acetone		Solvent, ethereal, apple, pear
630	11.11 (5)	Diacetyl	X	Butter
731	15.00 (9)	2-Pentanone	X	Ether, fruit
1034	26.78 (19)	6-Methyl-5-hepten-2-one	X	Pepper, mushroom, rubber
1128	30.05 (30)	Acetophenone	X	Must, flower, almond
	Alcohols			
-	7.26 (1)	Ethanol	X	Alcohol
655	12.06 (7)	2-methyl-3-buten-2-ol		Fruity
	Terpenes			
957	23.97 (16)	α-Pinene	X	Pine, turpentine
1051	27.40 (22)	D-Limonene	X	Citrus, mint
1054	27.50 (23)	*β*-Cymene	X	Solvent, gasoline, citrus
1064	27.85 (24)	Eucalyptol	X	Mint, sweet
1075	28.25 (25)	γ-Terpinene		Lemon
1101	29.15 (27)	Terpilonene		Sweet, fresh, piney citrus
1124	29.90 (29)	Linalool L	X	Flower, lavender
1192	32.14 (34)	Terpinen-4-ol		Turpentine, nutmeg, must
	Esters			
639	11.46 (6)	Ethyl acetate	X	Pineapple
711	14.16 (8)	Methyl isobutyrate		Etherial, diffusive, fruity, sweet
749	15.71 (11)	Methyl butanoate		Ether, fruit, sweet
1136	30.30 (31)	Methyl benzoate	X	Brune, lettuce, herb, sweet
1150	30.76 (32)	Benzyl acetate		Fresh, boiled vegetable
1188	32.00 (33)	Ethyl benzoate	X	Camomile, flower, celery, fruit
	Other			
-	8.70 (3)	Acetylhydrazine		
599	9.93 (4)	Hexane		Alkane
803	17.94 (12)	4-methyl-2-pentanol (ISTD)		
961	23.06 (14)	Styrene	X	Balsamic, gasoline
-	24,54 (17)	Octamethylcyclotetrasiloxane		

[A]ODOUR DESCRIPTIONS FROM FLAVORNET (ACREE AND ARN, 2004), [B] X = FOUND
[C]RETENTION INDEX

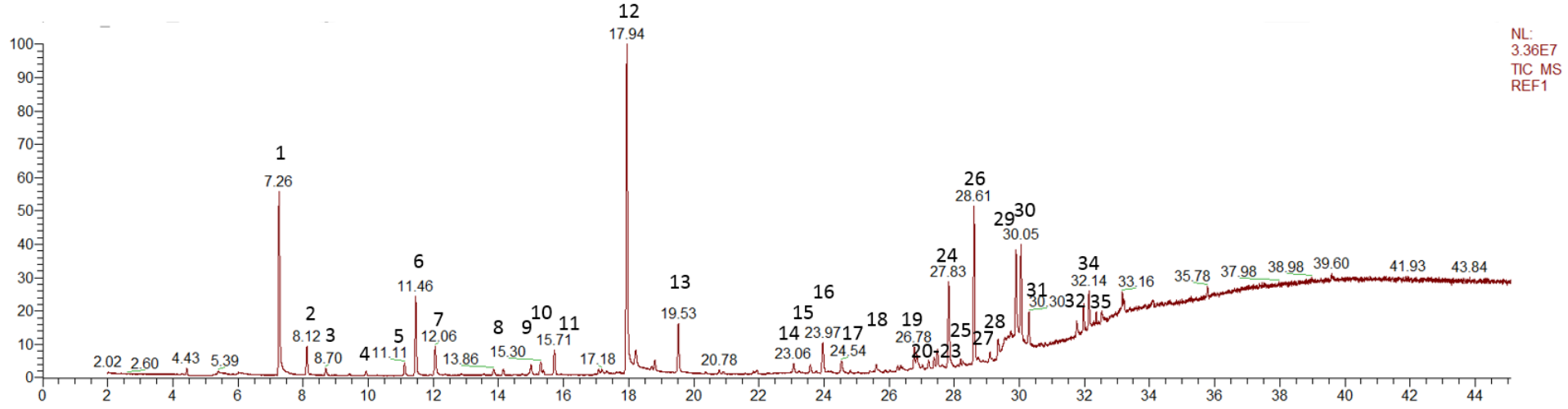

Figure 17. GC/MS chromatogram of lingonberry juice “without enzyme & heat” sample.

The retention times and numbers refer to peaks listed in table 3. Peak 12 is internal standard, 4-methyl-2-pentanol. The following ten peaks were not taken into comparison of enzymatic treatment: 18 ((Z)-2-Heptenal), 22 (D-Limonene), 25 (*γ*-Terpinene), 27 (Terpinolene), 28 (2 Octenal), 30 (Acetophenone), 31 (methyl benzoate), 32 (Benzyl acetate), 34 (Terpinen-4-ol), and 35 (Decanal) due to poor separation or insufficient identification.

7.2 Comparison of enzymatic treatments

Principal component analysis was used to study the data collected from GS/MS analysis of lingonberry juice volatiles.

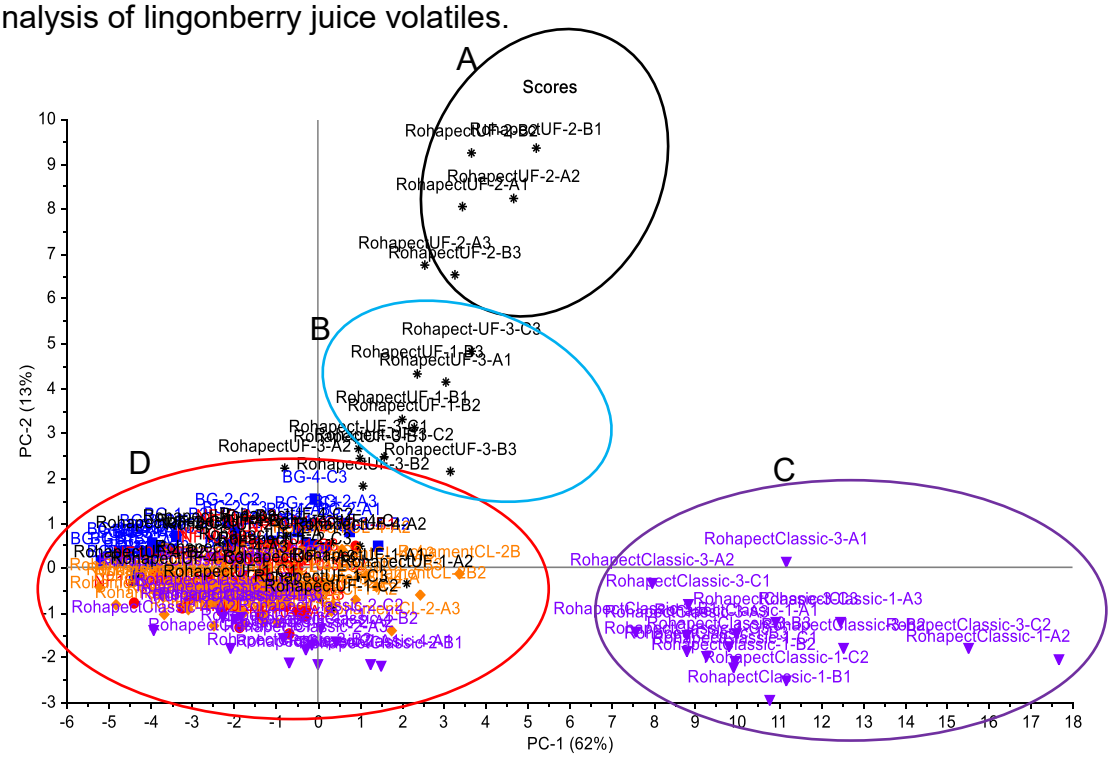

Figure 18. Scores of PCA model for lingonberry juice samples (n=197 x 5 samples) classified according to volatile contents (n=25). The colours signify the different enzymatic treatments.

From PCA four distinct separate groups can be identified. Group A consists of samples treated with Rohapect UF (RohapectUF-2) minimum dosage and 3-hour incubation. Group B consists of samples treated with Rohapect UF (RohapectUF-3/1) 1-hour incubations. Group C consists of samples treated with Rohapect Classic (RohapectClassic-1/3) 1-hour incubations. Group D consists of all the rest treatments including samples treated with Rohapect UF (Rohapect UF 4) and Rohapect Classic (Rohapect Classic 2/4).

In tables 4 - 9 the normalized peak areas (compound area/ISTD area; average ± SD) of volatile flavour compounds of lingonberry juices are represented with different enzymatic treatments. The impact of enzyme concentration and incubation time were studied with each treatment.

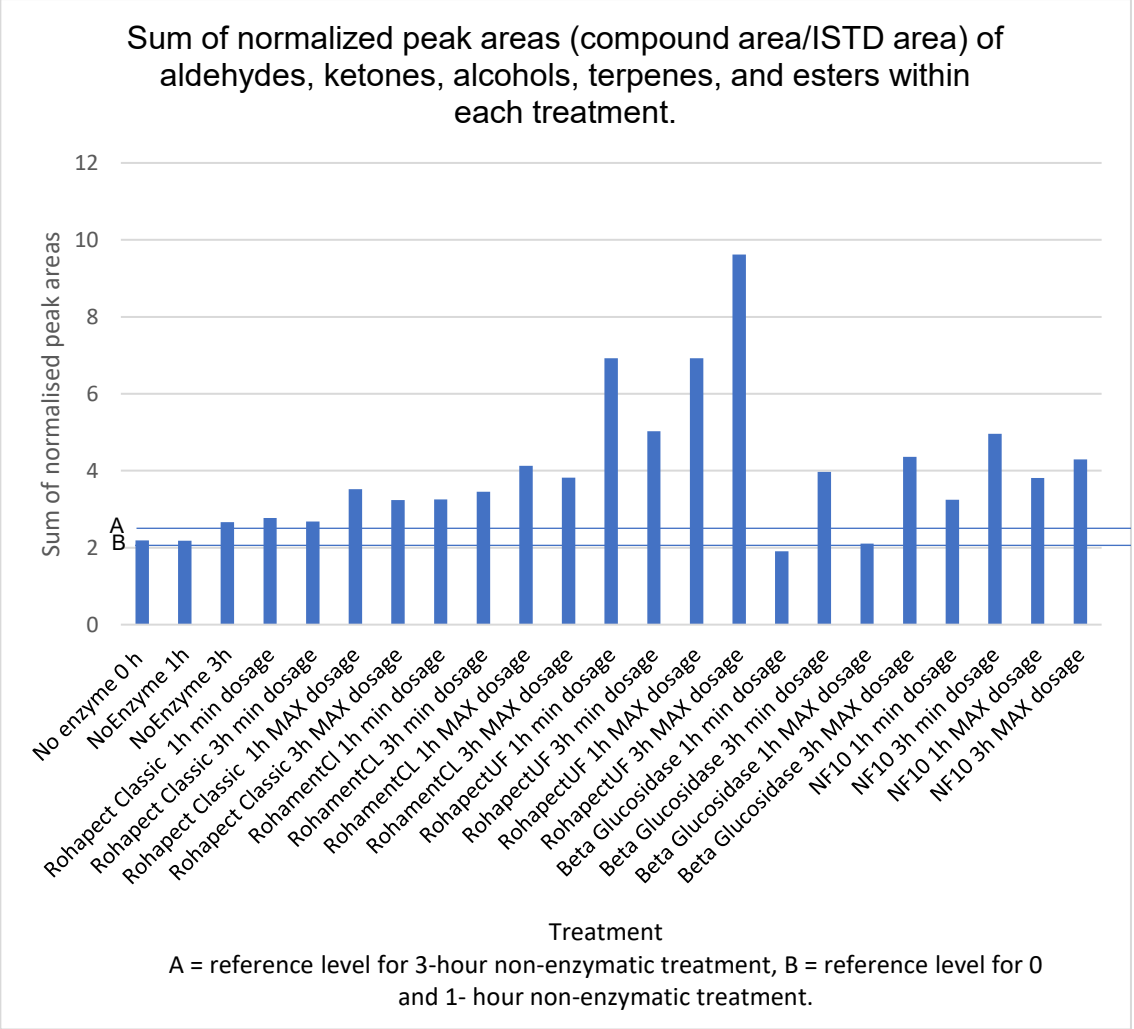

Figure 19. Sum of normalized peak areas (compound area/ISTD area) of aldehydes, ketones, alcohols, terpenes, and esters within each treatment.

Figure 19 visualises the impact of studied treatments on the total concentration of VOCs. Rohapect UF treatment can be singled out as resulting in largest increase in total VOCs. Beta Glucosidase treatments with minimum dosage resulted in decrease in total VOCs. Otherwise the enzymatic treatments result in increases when compared with non-enzymatic treatments, regardless of incubation time.

7.2.1 Non-enzymatic treatments

With these samples the only changing factor was the incubation time. Incubation time of 3-hours increased the total concentration of VOCs, whereas the difference between 0-hour and 1-hour incubation times are negligible.

Aldehyde concentrations increased along with increased incubation time. Diacetyl concentrations also increased with incubation time, which might have an unsatisfactory impact on the flavour. *α*-Pinene concentrations were higher with 1-hour incubation than with 3-hour incubation, which was the only compound with this behaviour. Ester concentrations either decreased or stayed relatively same with incubations. As esters are linked to sweet flavours, this decrease might have an impact on the observed sweetness of the juices.

Table 4. Normalized peak areas (compound area/ISTD area; average ± SD) of volatile compounds of lingonberry juices with non-enzymatic treatment.

Compound	No enzyme, no heat (REF)	No enzyme, 1h incubation	No enzyme, 3h incubation
Aldehydes			
Pentanal	0.056±0.001	0.068±0.01	0.083±0.018
Hexanal	0.066±0.005	0.097±0.022	0.114±0.015
Heptanal	0.009±0.001	0.01±0.003	0.01±0.002
Benzaldehyde	0.024±0.001	0.059±0.015	0.12±0.015
Octanal	0.005±0.001	0.011±0.006	0.011±0.004
Ketones			
Acetone	0.13±0.005	0.082±0.012	0.112±0.015
Diacetyl	0.066±0.002	0.129±0.021	0.157±0.02
2-Pentanone	0.042±0.001	0.043±0.005	0.058±0.007
6-Methyl-5-hepten-2-one	0.024±0.001	0.03±0.006	0.033±0.006
Alcohols			
Ethanol	0.943±0.006	0.831±0.131	1.013±0.156
2-methyl-3-buten-2-ol	0.092±0.004	0.079±0.019	0.107±0.025
Terpenes			
α-Pinene	0.07±0.004	0.182±0.082	0.132±0.027
β-Cymene	0.036±0.016	0.043±0.017	0.049±0.008
Eucalyptol	0.057±0.004	0.047±0.013	0.044±0.007
Linalool L	0.104±0.012	0.133±0.033	0.231±0.04
Esters			
Ethyl acetate	0.332±0.005	0.239±0.036	0.248±0.029
Methyl isobutyrate	0.011±0	0.011±0.001	0.013±0.003
Methyl butanoate	0.047±0.002	0.049±0.007	0.058±0.007
Ethyl benzoate	0.075±0.004	0.042±0.011	0.068±0.012
Other			
Acetylhydrazine	0.031±0.001	0.028±0.005	0.032±0.004
Hexane	0.009±0.001	0.02±0.003	0.02±0.003
Styrene	0.025±0.004	0.01±0.005	0.01±0.004
Octamethylcyclotetrasiloxane	0.057±0.012	0.081±0.018	0.108±0.041
Undecane	0.246±0.015	0.239±0.031	0.29±0.054

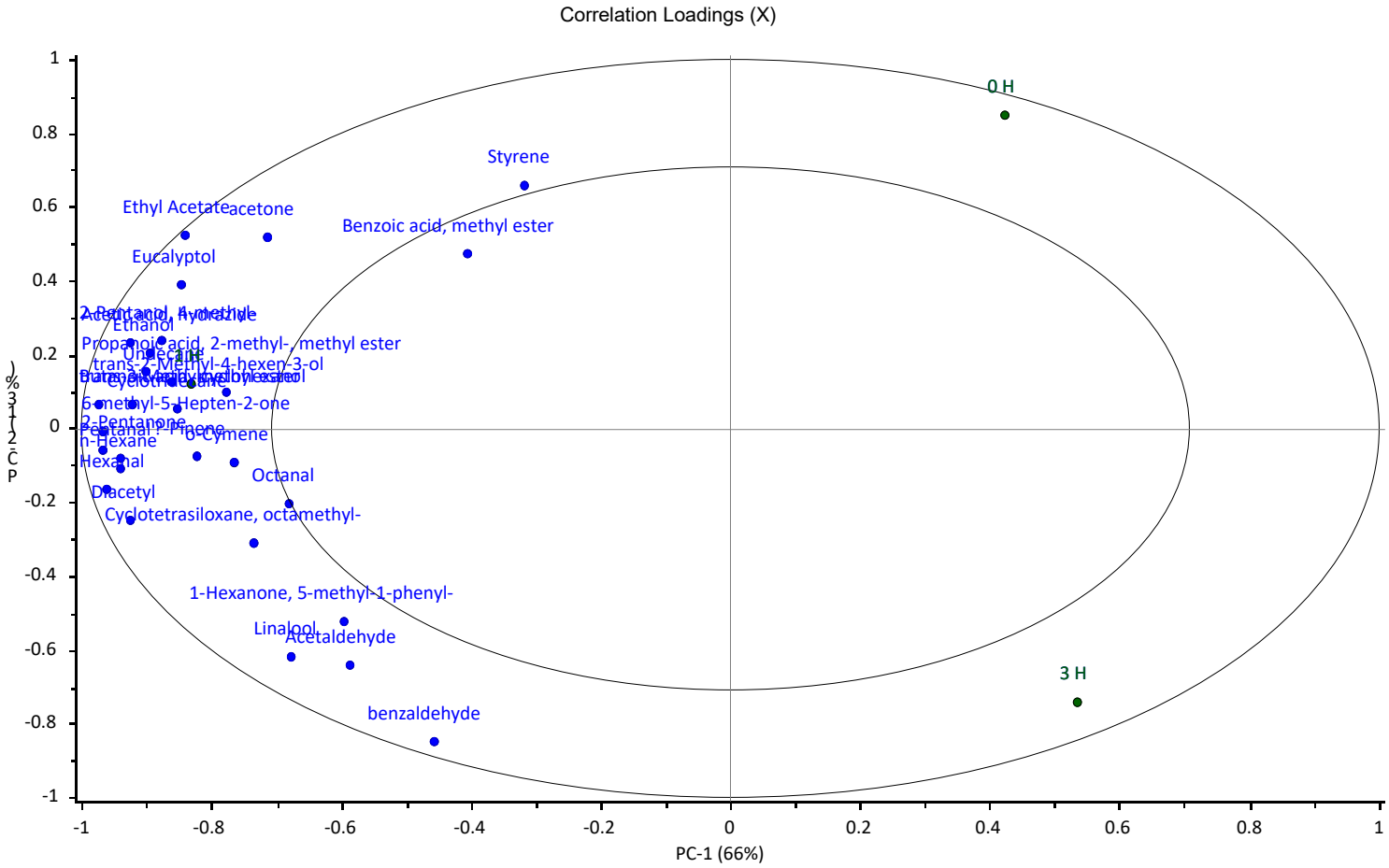

Figure 20. PCA model for lingonberry juices with non-enzymatic treatment (n=36 samples) classified according to volatile contents (n=24)

7.2.2 Rohapect Classic treatment

Maximum dosage resulted in increase of total concentration of VOCs, whereas longer incubation time decreased the total concentration of VOCs.

With Rohapect Classic enzymatic treatment the concentration of aldehydes generally increased. The concentration of octanal didn't see significant changes. Hexanal and Benzaldehyde concentrations doubled with all treatments. Ketones diacetyl and 2-pentanone increased their concentrations with maximum dosages. Acetone levels decreased with minimum dosages. Terpenes decreased with all treatments, except linalool L, which saw significant increase in concentration with all treatments. The decrease was slighter with 1-hour max dosage treatment. Ester concentrations decreased more with minimum dosage. Ethyl acetate concentration grew with maximum dosages.

The changes in total concentrations are slight and varied, as can be deducted from the increases and decreases with different treatments. The impact of increased linalool L concentrations might have a slight impact on flowery flavour of the juices. On the other hand, it is a moot point to speculate without sensory evaluation. Octanal is the only compound in this study that Viljanen et al. (2014) refer to as having impact on sensory evaluations, and its concentrations did not change with this treatment. The increased diacetyl concentration might cause unsavoury flavour. In PCA model the impact of incubation time explains 82 % of differences in samples.

Table 5. Normalized peak areas (compound area/ISTD area; average ± SD) of volatile compounds of lingonberry juices with Rohapect Classic treatment.

Compound	REF	Rohapect Classic 1 1h min dosage	Rohapect Classic 2 3h min dosage	Rohapect Classic 3 1h MAX dosage	Rohapect Classic 4 3h MAX dosage
Aldehydes					
Pentanal	0.056±0.001	0.071±0.006	0.064±0.002	0.086±0.013	0.081±0.018
Hexanal	0.066±0.005	0.115±0.015	0.111±0.052	0.141±0.028	0.122±0.023
Heptanal	0.009±0.001	0.008±0.002	0.008±0.004	0.01±0.002	0.007±0.002
Benzaldehyde	0.024±0.001	0.082±0.01	0.101±0.051	0.08±0.01	0.111±0.03
Octanal	0.005±0.001	0.006±0.002	0.005±0.002	0.008±0.002	0.005±0.001
Ketones					
Acetone	0.13±0.005	0.092±0.014	0.116±0.061	0.131±0.025	0.148±0.039
Diacetyl	0.066±0.002	0.095±0.01	0.089±0.045	0.118±0.025	0.109±0.027
2-Pentanone	0.042±0.001	0.044±0.004	0.044±0.009	0.053±0.007	0.054±0.006
6-Methyl-5-hepten-2-one	0.024±0.001	0.036±0.006	0.031±0.015	0.038±0.007	0.035±0.007
Alcohols					
Ethanol	0.943±0.006	1.403±0.238	1.237±0.583	1.784±0.334	1.53±0.391
2-methyl-3-buten-2-ol	0.092±0.004	0.132±0.009	0.145±0.085	0.162±0.029	0.184±0.057
Terpenes					
α-Pinene	0.07±0.004	0.025±0.006	0.026±0.019	0.044±0.029	0.022±0.02
β-Cymene	0.036±0.016	0.026±0.007	0.03±0.015	0.038±0.01	0.029±0.01
Eucalyptol	0.057±0.004	0.038±0.005	0.03±0.012	0.047±0.008	0.034±0.008
Linalool L	0.104±0.012	0.185±0.018	0.223±0.127	0.216±0.04	0.243±0.042
Esters					
Ethyl acetate	0.332±0.005	0.307±0.064	0.287±0.1	0.422±0.08	0.369±0.079
Methyl isobutyrate	0.011±0	0.013±0.001	0.025±0.006	0.015±0.002	0.022±0.008
Methyl butanoate	0.047±0.002	0.037±0.008	0.043±0.009	0.048±0.008	0.051±0.005
Ethyl benzoate	0.075±0.004	0.058±0.005	0.062±0.026	0.079±0.011	0.082±0.015
Other					
Acetylhydrazine	0.031±0.001	0.034±0.009	0.035±0.017	0.055±0.011	0.051±0.012
Hexane	0.009±0.001	0.015±0.002	0.013±0.003	0.016±0.003	0.016±0.003
Styrene	0.025±0.004	0.013±0.005	0.011±0.008	0.01±0.004	0.009±0.002
Octamethylcyclotetrasiloxane	0.057±0.012	0.063±0.026	0.068±0.024	0.077±0.037	0.096±0.05
Undecane	0.246±0.015	0.222±0.016	0.211±0.039	0.176±0.017	0.215±0.018

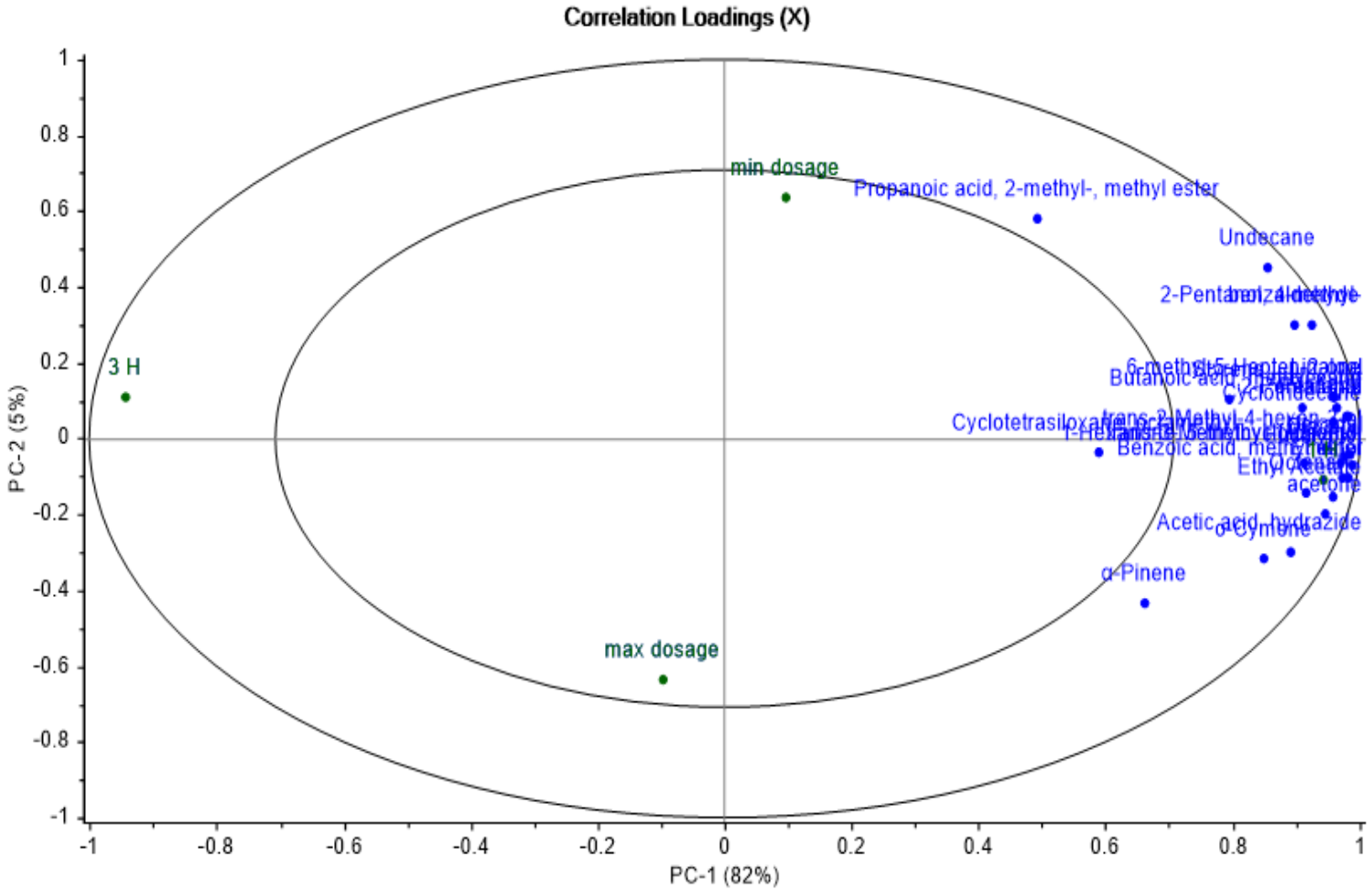

Figure 21. PCA model for Rohapect Classic treated lingonberry juices (n=36 samples) classified according to volatile contents (n=24).

7.2.3 Rohament CL treatment

All treatments with Rohament CL resulted in increase in total VOCs. Maximum dosage had bigger impact than longer incubation time.

With Rohament CL enzymatic treatment the concentration of all aldehydes increased. Pentanal and hexanal increased their concentration the most with 1-hour maximum dosage treatment. Benzaldehyde increased in concentration the most with 3-hour maximum dosage treatment. All ketones increased in concentration with all treatments. Acetone and diacetyl increased the most in concentration with 3-hour maximum dosage treatment. All terpenes except eucalyptol increased in concentration with all treatments. Eucalyptol concentrations decreased slightly with 3-hour incubation times. Other terpenes increased the most in concentration with 3-hourincubation times. All ester, except ethyl benzoate, concentrations increased with all treatments. 1-hour maximum dosage yielded the highest increases in concentrations.

These overall increases could have an impact on the overall strength of the flavour sensation. The increased diacetyl concentration might cause unsavoury flavour. In PCA model the impact of incubation time explains 62% of differences in samples

Table 6. Normalized peak areas (compound area/ISTD area; average ± SD) of volatile compounds of lingonberry juices with Rohament CL treatment.

Compound	REF	RohamentCl 1 1h min dosage	RohamentC L 2 3h min dosage	RohamentCL 3 1h MAX dosage	RohamentCL 4 3h MAX dosage
Aldehydes					
Pentanal	0.056±0.001	0.089±0.02	0.084±0.02	0.125±0.017	0.092±0.038
Hexanal	0.066±0.005	0.176±0.033	0.184±0.042	0.245±0.046	0.21±0.099
Heptanal	0.009±0.001	0.016±0.006	0.014±0.004	0.017±0.005	0.018±0.007
Benzaldehyde	0.024±0.001	0.105±0.023	0.131±0.027	0.109±0.028	0.152±0.06
Octanal	0.005±0.001	0.014±0.004	0.009±0.002	0.016±0.003	0.012±0.004
Ketones					
Acetone	0.13±0.005	0.099±0.02	0.179±0.056	0.154±0.035	0.203±0.07
Diacetyl	0.066±0.002	0.121±0.023	0.129±0.048	0.141±0.046	0.159±0.031
2-Pentanone	0.042±0.001	0.053±0.007	0.052±0.007	0.065±0.009	0.058±0.007
6-Methyl-5-hepten-2-one	0.024±0.001	0.04±0.008	0.045±0.011	0.055±0.011	0.054±0.022
Alcohols					
Ethanol	0.943±0.006	1.438±0.248	1.517±0.364	1.824±0.437	1.579±0.615
2-methyl-3-buten-2-ol	0.092±0.004	0.168±0.034	0.178±0.032	0.207±0.051	0.191±0.058
Terpenes					
α-Pinene	0.07±0.004	0.131±0.038	0.121±0.045	0.149±0.058	0.179±0.051
β-Cymene	0.036±0.016	0.057±0.009	0.056±0.009	0.064±0.014	0.065±0.027
Eucalyptol	0.057±0.004	0.058±0.01	0.048±0.008	0.067±0.011	0.053±0.016
Linalool L	0.104±0.012	0.18±0.037	0.174±0.032	0.213±0.038	0.219±0.063
Esters					
Ethyl acetate	0.332±0.005	0.359±0.053	0.405±0.091	0.504±0.101	0.412±0.174
Methyl isobutyrate	0.011±0	0.037±0.004	0.023±0.01	0.041±0.005	0.033±0.009
Methyl butanoate	0.047±0.002	0.057±0.008	0.058±0.009	0.071±0.009	0.063±0.009
Ethyl benzoate	0.075±0.004	0.055±0.01	0.045±0.018	0.06±0.009	0.064±0.022
Other					
Acetylhydrazine	0.031±0.001	0.033±0.006	0.049±0.011	0.056±0.012	0.053±0.018
Hexane	0.009±0.001	0.021±0.004	0.023±0.004	0.027±0.005	0.027±0.005
Styrene	0.025±0.004	0.039±0.014	0.023±0.01	0.036±0.016	0.032±0.029
Octamethylcyclotetrasiloxane	0.057±0.012	0.067±0.013	0.056±0.014	0.065±0.011	0.083±0.077
Undecane	0.246±0.015	0.379±0.033	0.35±0.054	0.351±0.022	0.322±0.06

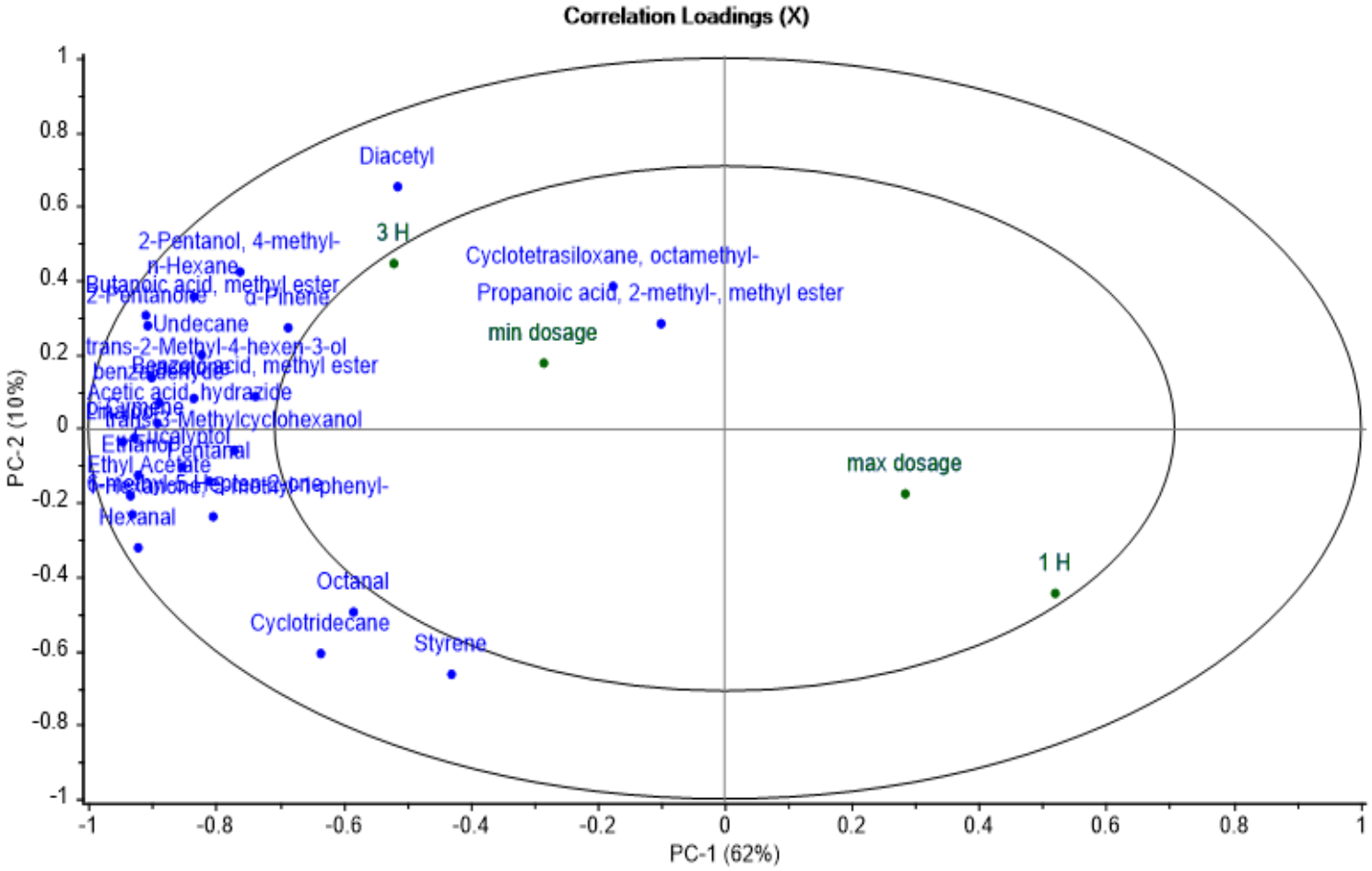

Figure 22. PCA model for Rohament CL treated lingonberry juices (n=36 samples) classified according to volatile contents (n=24)

7.2.4 Rohapect UF treatment

Generally, the normalised peak areas are clearly the highest with Rohapect UF treatment when compared with all treatments. With maximum dosage, longer incubation time yields larger total concentration of VOCs. With minimum dosage, shorter incubation time yields more total VOCs. This could be a possible mistake in the labelling of the data (sample sets 1 & 2 were mixed possibly), or otherwise this phenomenon should be looked in to closer in following studies.

All aldehydes grew in concentration. Hexanal and benzaldehyde grew in concentration significantly. All ketones grew in concentration, the most with 3-hour maximum dosage treatment. All terpenes grew in concentration. 1-hour incubation time favoured the increase with minimum dosage, and 3-hour incubation with maximum dosage. All esters grew in concentration.

The significantly increased benzaldehyde and diacetyl concentrations might cause unsatisfactory flavour. On the other hand, the general increase in all treatments might lead to overall stronger lingonberry flavour sensation. In PCA model the impact of incubation time explains 43 % of differences in samples

Table 7. Normalized peak areas (compound area/ISTD area; average ± SD) of volatile compounds of lingonberry juices with Rohapect UF treatment.

Compound	REF	RohapectUF 4 1h min dosage	RohapectUF 1 3h min dosage	RohapectUF 2 1h MAX dosage	RohapectUF 3 3h MAX dosage
Aldehydes					
Pentanal	0.056±0.001	0.16±0.102	0.107±0.025	0.103±0.015	0.161±0.094
Hexanal	0.066±0.005	0.365±0.253	0.191±0.068	0.195±0.094	0.171±0.111
Heptanal	0.009±0.001	0.02±0.01	0.014±0.005	0.017±0.005	0.016±0.013
Benzaldehyde	0.024±0.001	0.204±0.113	0.229±0.133	0.368±0.18	0.591±0.386
Octanal	0.005±0.001	0.016±0.012	0.012±0.003	0.011±0.004	0.015±0.009
Ketones					
Acetone	0.13±0.005	0.256±0.174	0.199±0.049	0.185±0.082	0.334±0.185
Diacetyl	0.066±0.002	0.231±0.151	0.17±0.054	0.236±0.074	0.343±0.207
2-Pentanone	0.042±0.001	0.099±0.059	0.072±0.015	0.07±0.013	0.105±0.062
6-Methyl-5-hepten-2-one	0.024±0.001	0.099±0.057	0.045±0.01	0.061±0.021	0.061±0.032
Alcohols					
Ethanol	0.943±0.006	3.005±1.927	2.183±0.619	2.866±0.975	4.043±2.493
2-methyl-3-buten-2-ol	0.092±0.004	0.373±0.211	0.359±0.158	0.497±0.204	0.909±0.486
Terpenes					
α-Pinene	0.07±0.004	0.211±0.171	0.169±0.064	0.379±0.189	0.266±0.229
β-Cymene	0.036±0.016	0.12±0.091	0.084±0.021	0.117±0.036	0.132±0.093
Eucalyptol	0.057±0.004	0.116±0.075	0.063±0.023	0.126±0.042	0.134±0.082
Linalool L	0.104±0.012	0.511±0.253	0.274±0.065	0.257±0.065	0.378±0.197
Esters					
Ethyl acetate	0.332±0.005	0.808±0.524	0.656±0.272	1.186±0.478	1.596±0.971
Methyl isobutyrate	0.011±0	0.023±0.016	0.029±0.019	0.009±0.009	0.063±0.035
Methyl butanoate	0.047±0.002	0.098±0.065	0.069±0.017	0.063±0.016	0.091±0.056
Ethyl benzoate	0.075±0.004	0.212±0.122	0.098±0.028	0.178±0.044	0.212±0.115
Other					
Acetylhydrazine	0.031±0.001	0.099±0.069	0.067±0.023	0.11±0.038	0.157±0.094
Hexane	0.009±0.001	0.022±0.016	0.021±0.006	0.014±0.004	0.029±0.019
Styrene	0.025±0.004	0.063±0.02	0.049±0.035	0.158±0.084	0.11±0.096
Octamethylcyclotetrasiloxane	0.057±0.012	0.168±0.11	0.211±0.114	0.153±0.117	0.231±0.201
Undecane	0.246±0.015	0.323±0.208	0.383±0.079	0.26±0.03	0.47±0.327

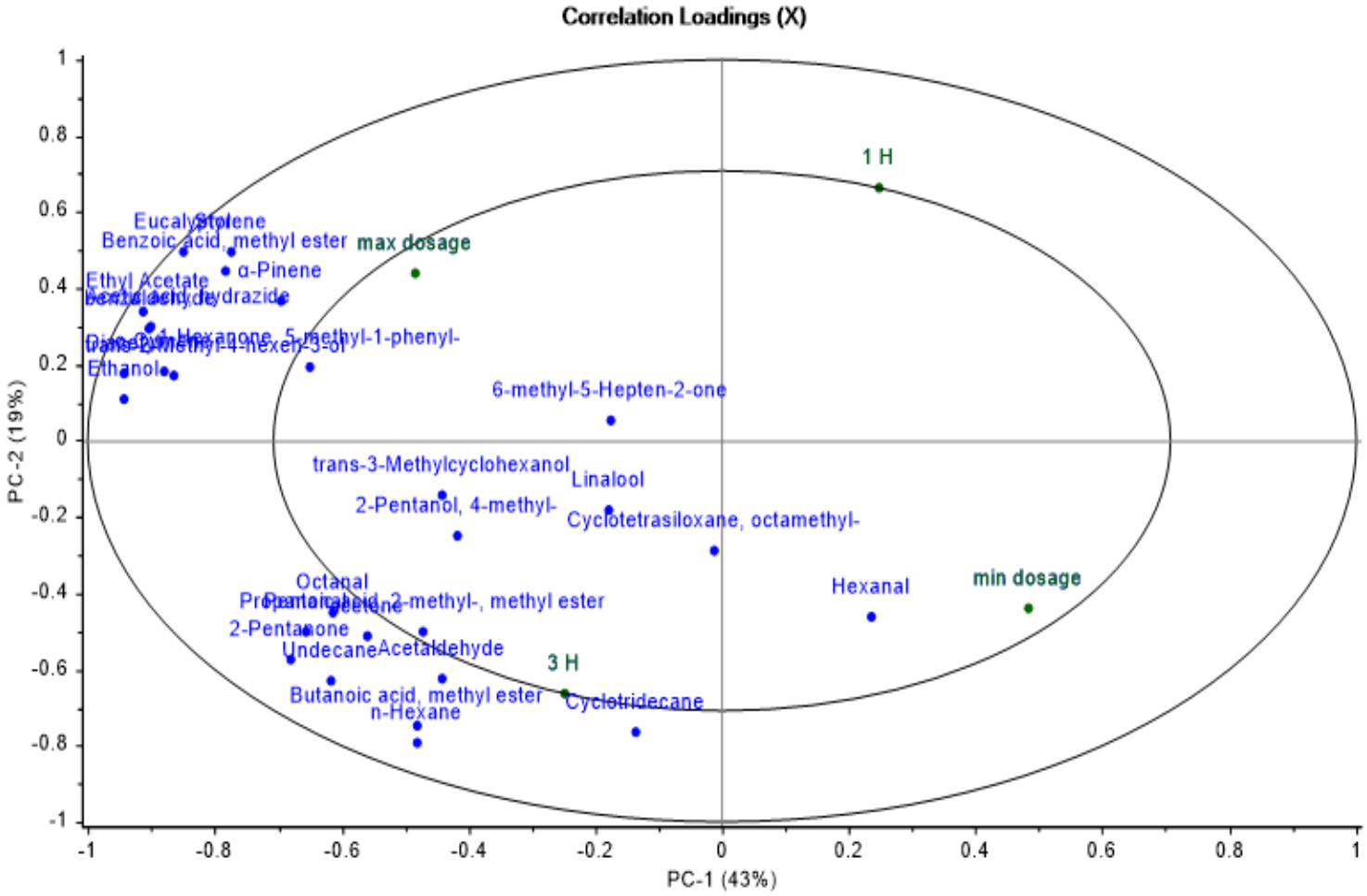

Figure 23. PCA model for Rohapect UF treated lingonberry juices (n=36 samples) classified according to volatile contents (n=24).

7.2.5 Beta glucosidase treatment

Beta glucosidase treatment is the only one where the total concentration of VOCs decreases with both 1-hour incubation time treatments.

On average when comparing the 3-hour incubation and 1-hour incubation, the normalised peak areas are twice as large in the 3-hour incubation group. By percentage in the aldehyde group the difference is on average 50.9 %, in the ketone group 56.4 %, in the alcohol group 59.4 %, in the terpene group 30.2 %, and in the ester group 48.2 %. Figure 24 highlights this clear impact of incubation time. Sensory evaluations for beta glucosidase treated juices with different incubation times would be of most interest, as the differences in the concentrations are the clearest with this treatment. My hypothesis is that the general lingonberry like flavour is identifiably stronger with samples with the 3-hour incubation time.

In PCA model the impact of incubation time explains 65 % of differences in samples, meaning incubation time is the dominant factor concerning VOC concentration. Dosage seems to have no impact on normalized peak areas of volatile compounds of lingonberry juices with the Beta Glucosidase treatment

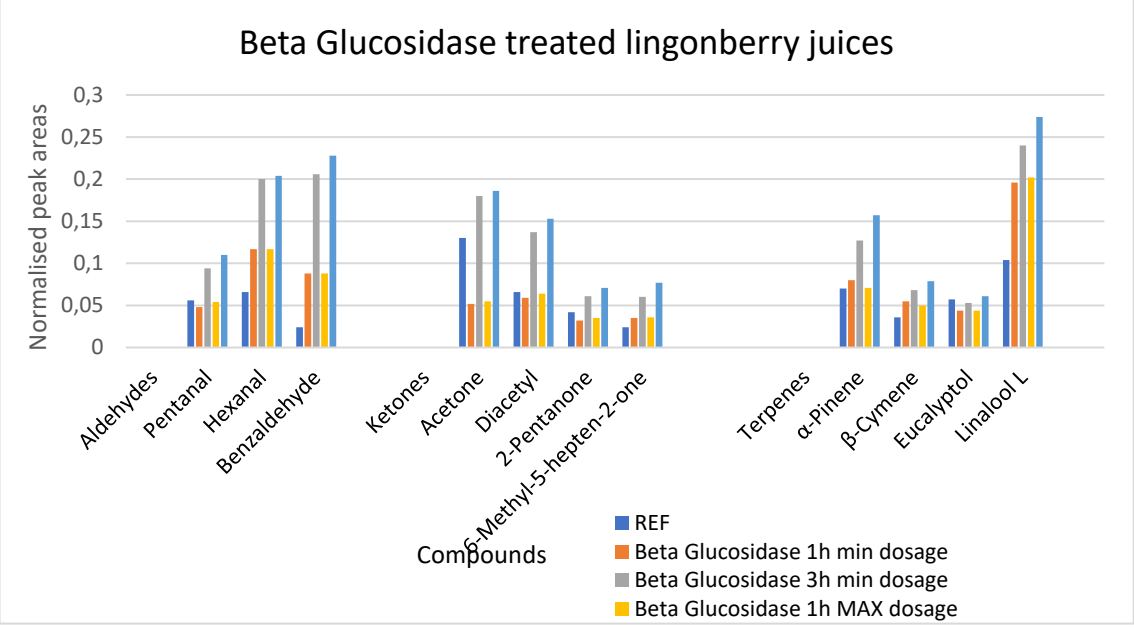

Figure 24. The clear impact of incubation time with beta glucosidase treatment with selected compounds (n = 11).

Table 8. Normalised peak areas (compound area/ISTD area; average ± SD) of volatile compounds of lingonberry juices with Beta Glucosidase treatment.

Compound	REF	Beta Glucosidase 1 1h min dosage	Beta Glucosidase 2 3h min dosage	Beta Glucosidase 3 1h MAX dosage	Beta Glucosidase 4 3h MAX dosage
Aldehydes					
Pentanal	0.056±0.001	0.048±0.008	0.094±0.031	0.054±0.003	0.11±0.025
Hexanal	0.066±0.005	0.117±0.031	0.2±0.053	0.117±0.019	0.204±0.034
Heptanal	0.009±0.001	0.01±0.003	0.015±0.007	0.009±0.002	0.016±0.004
Benzaldehyde	0.024±0.001	0.088±0.017	0.206±0.075	0.088±0.019	0.228±0.04
Octanal	0.005±0.001	0.007±0.002	0.011±0.004	0.006±0.001	0.011±0.003
Ketones					
Acetone	0.13±0.005	0.052±0.012	0.18±0.057	0.055±0.008	0.186±0.038
Diacetyl	0.066±0.002	0.059±0.015	0.137±0.046	0.064±0.012	0.153±0.029
2-Pentanone	0.042±0.001	0.032±0.002	0.061±0.012	0.035±0.002	0.071±0.011
6-Methyl-5-hepten-2-one	0.024±0.001	0.035±0.008	0.06±0.022	0.036±0.005	0.077±0.022
Alcohols					
Ethanol	0.943±0.006	0.694±0.17	1.671±0.637	0.838±0.128	1.731±0.386
2-methyl-3-buten-2-ol	0.092±0.004	0.092±0.027	0.236±0.101	0.097±0.028	0.285±0.046
Terpenes					
α-Pinene	0.07±0.004	0.08±0.024	0.127±0.018	0.071±0.023	0.157±0.021
β-Cymene	0.036±0.016	0.055±0.012	0.068±0.013	0.05±0.007	0.079±0.014
Eucalyptol	0.057±0.004	0.044±0.011	0.053±0.008	0.044±0.006	0.061±0.011
Linalool L	0.104±0.012	0.196±0.035	0.24±0.039	0.202±0.036	0.274±0.056
Esters					
Ethyl acetate	0.332±0.005	0.202±0.042	0.474±0.157	0.239±0.039	0.551±0.105
Methyl isobutyrate	0.011±0	0.004±0.002	0.012±0.002	0.006±0	0.018±0.004
Methyl butanoate	0.047±0.002	0.027±0.004	0.052±0.009	0.03±0.003	0.066±0.012
Ethyl benzoate	0.075±0.004	0.064±0.01	0.073±0.018	0.062±0.008	0.08±0.018
Other					
Acetylhydrazine	0.031±0.001	0.02±0.004	0.048±0.015	0.027±0.004	0.058±0.012
Hexane	0.009±0.001	0.002±0	0.007±0.001	0.003±0.001	0.012±0.004
Styrene	0.025±0.004	0.029±0.007	0.046±0.018	0.026±0.012	0.057±0.013
Octamethylcyclotetrasiloxane	0.057±0.012	0.168±0.098	0.266±0.298	0.197±0.207	0.116±0.035
Undecane	0.246±0.015	0.065±0.021	0.239±0.056	0.082±0.029	0.366±0.1

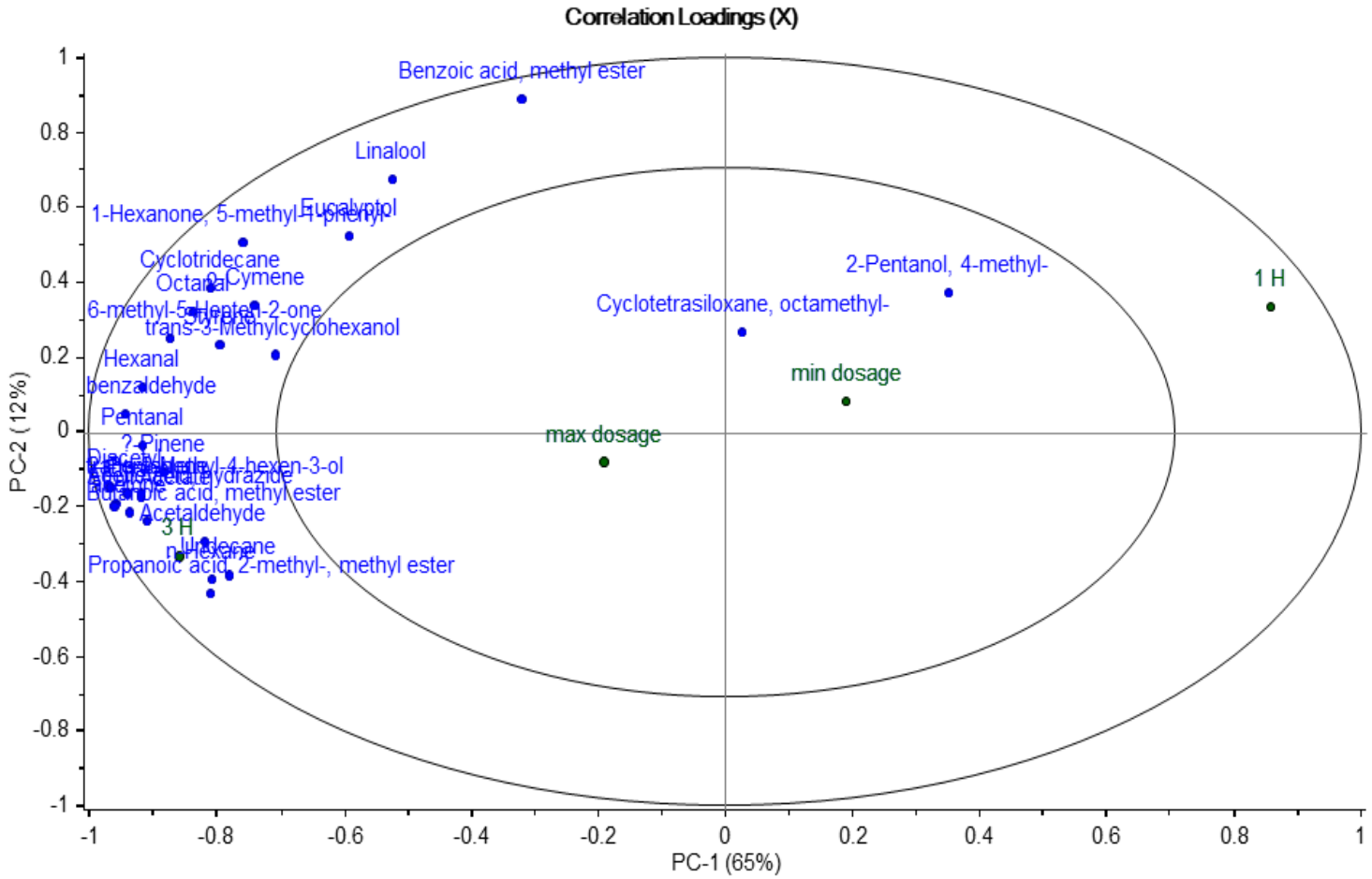

Figure 25. PCA model for Beta Glucosidase treated lingonberry juices (n=36 samples) classified according to volatile contents (n=24).

7.2.6 NF10 treatment

The impact on total concentration of VOCs is hard to attribute to neither incubation time or enzyme dosage.

On average when comparing the 3-hour incubation and 1-hour incubation the normalised peak areas are larger in the 3-hour incubation group. Interestingly in the 3-hour incubation the higher dosage seems to reduce the concentration of measured volatiles, as in the 1-hour incubation the higher dosage increases the concentration of measured volatiles.

All aldehydes, ketones, and terpenes grew in concentration with all treatments, more with 3-hour incubation time. Esters grew in concentration the most with 3-hour incubation with minimum dosage. Ethyl benzoate decreased slightly in concentration, except with 1-hour maximum dosage treatment.

These overall increases could have an impact on the overall strength of the flavour sensation.

Table 9. Normalized peak areas (compound area/ISTD area; average ± SD) of volatile compounds of lingonberry juices with NF10 treatment.

Compound	REF	NF10 1 1h min dosage	NF10 2 3h min dosage	NF10 3 1h MAX dosage	NF10 4 3h MAX dosage
Aldehydes					
Pentanal	0.056±0.001	0.087±0.023	0.147±0.054	0.111±0.021	0.122±0.039
Hexanal	0.066±0.005	0.189±0.019	0.255±0.066	0.22±0.038	0.224±0.056
Heptanal	0.009±0.001	0.015±0.003	0.023±0.008	0.018±0.005	0.018±0.007
Benzaldehyde	0.024±0.001	0.091±0.007	0.201±0.054	0.12±0.023	0.188±0.055
Octanal	0.005±0.001	0.01±0.001	0.014±0.004	0.013±0.002	0.011±0.003
Ketones					
Acetone	0.13±0.005	0.109±0.016	0.248±0.09	0.139±0.024	0.211±0.068
Diacetyl	0.066±0.002	0.109±0.018	0.249±0.111	0.15±0.026	0.19±0.071
2-Pentanone	0.042±0.001	0.06±0.006	0.097±0.028	0.072±0.011	0.081±0.02
6-Methyl-5-hepten-2-one	0.024±0.001	0.046±0.005	0.065±0.017	0.062±0.012	0.061±0.014
Alcohols					
Ethanol	0.943±0.006	1.361±0.229	1.973±0.671	1.549±0.431	1.661±0.557
2-methyl-3-buten-2-ol	0.092±0.004	0.17±0.022	0.3±0.09	0.193±0.034	0.256±0.088
Terpenes					
α-Pinene	0.07±0.004	0.138±0.026	0.195±0.056	0.161±0.033	0.183±0.046
β-Cymene	0.036±0.016	0.07±0.011	0.089±0.025	0.075±0.016	0.076±0.018
Eucalyptol	0.057±0.004	0.063±0.006	0.067±0.021	0.07±0.012	0.063±0.013
Linalool L	0.104±0.012	0.172±0.017	0.287±0.065	0.217±0.053	0.304±0.08
Esters					
Ethyl acetate	0.332±0.005	0.404±0.058	0.519±0.161	0.465±0.122	0.444±0.136
Methyl isobutyrate	0.011±0	0.016±0.002	0.064±0.016	0.019±0.003	0.047±0.017
Methyl butanoate	0.047±0.002	0.062±0.007	0.102±0.031	0.072±0.016	0.084±0.023
Ethyl benzoate	0.075±0.004	0.072±0.008	0.066±0.014	0.084±0.018	0.066±0.017
Other					
Acetylhydrazine	0.031±0.001	0.041±0.006	0.06±0.022	0.052±0.014	0.052±0.017
Hexane	0.009±0.001	0.011±0.001	0.029±0.008	0.012±0.002	0.024±0.008
Styrene	0.025±0.004	0.035±0.013	0.037±0.021	0.045±0.013	0.032±0.009
Octamethylcyclotetrasiloxane	0.057±0.012	0.093±0.029	0.361±0.263	0.14±0.128	0.32±0.083
Undecane	0.246±0.015	0.28±0.021	0.578±0.149	0.279±0.049	0.464±0.125

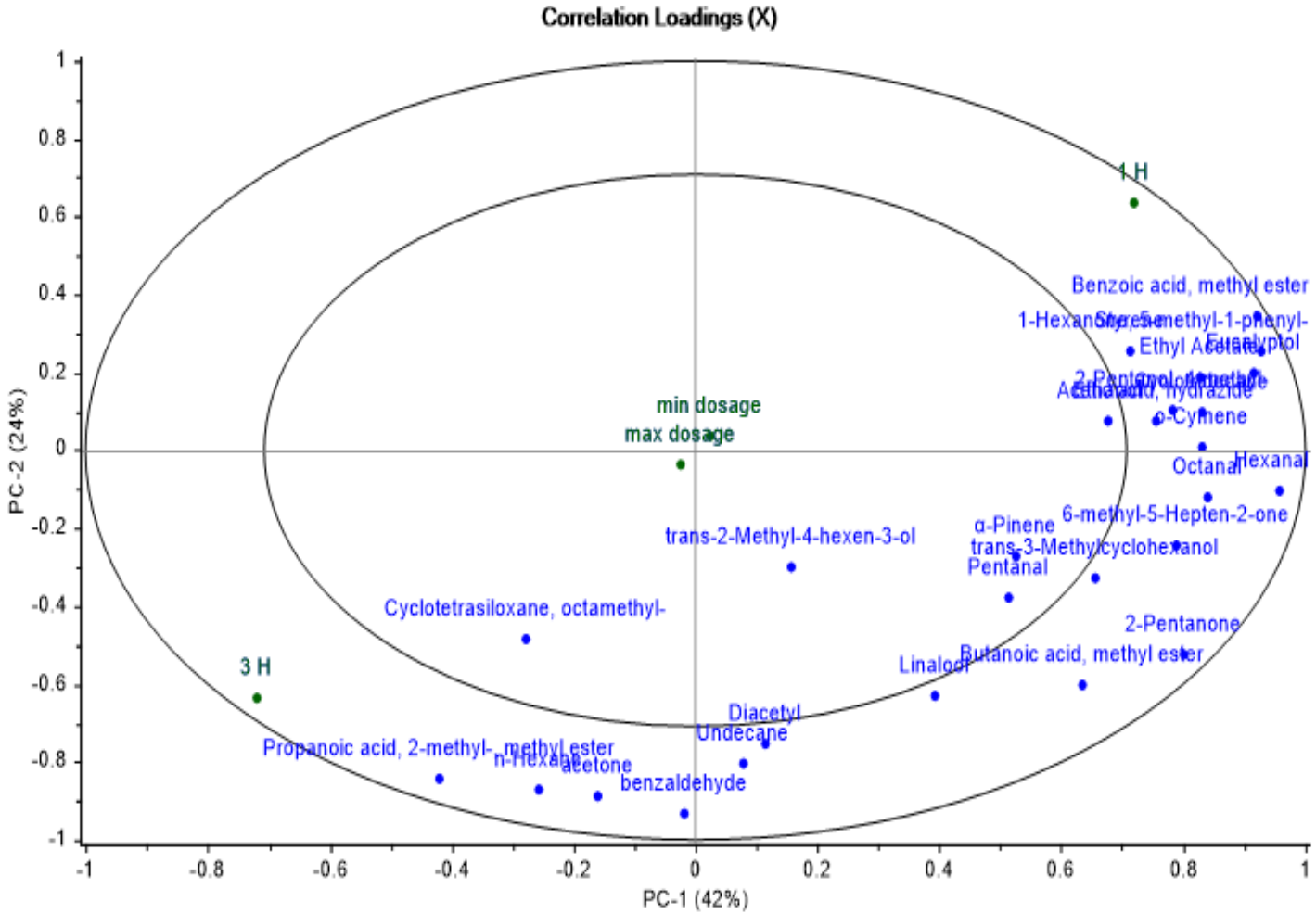

Figure 26. PCA model for NF10 treated lingonberry juices (n=36 samples) classified according to volatile contents (n=24).

7.3 Lingonberry juice yields

Generally higher enzyme concentration should result in better interaction between the enzyme and substrate, thus promoting plant cell wall solubilisation. The yields of juices treated with Beta Glucosidase and Rohapect UF behave according to this principle. The percentage difference between the lowest yield, which was the reference sample, and the highest yield, Rohapect Classic treatment with 3-hour incubation time with maximum dosage, is a mere 11.3.

The highest yields were obtained with Rohapect Classic treatment, with which the increased enzyme dosage and incubation time gave little more results.

Table 10. Yields of juice pressing with used treatments.

Treatment	Incubation time (h)	Enzyme dosage	Yield (%)
Without enzyme	3	0	63.3
	1	0	75.2
(REFERENCE)	0	0	69.9
Rohapect Classic	1	minimum	80.2
	1	maximum	80.6
	3	minimum	80.3
	3	maximum	81.2
Rohament CL	1	minimum	71.3
	1	maximum	73.7
	3	minimum	68.7
	3	maximum	68.7
Rohapect UF	1	minimum	74.1
	1	maximum	78.2
	3	minimum	72.9
	3	maximum	74.2
Beta Glucosidase	1	minimum	71.5
	1	maximum	75.6
	3	minimum	72.9
	3	maximum	77.3
NF 10	1	minimum	72.0
	1	maximum	72.4
	3	minimum	68.9
	3	maximum	70.2

As in a larger scale production prolonged incubation time may increase the solubilisation of plant cell wall components, but too long incubation time can lead to lesser energy efficiency and product quality, these small-scale results hardly justify tripling the incubation time. With Rohament CL, Rohapect UF, and NF 10 the longer incubation times resulted in lesser yields. According to these small-scale results, the best yield results are gained with Rohapect Classic treatment, as the minimum dosage and 1-hour incubation time lead to best results by efficiency.

8 Conclusions

Using the studied enzymes increased the total concentrations of VOCs all around more than just heat treatments, except with the special case of Beta Glucosidase, as seen in figure 19. Rohapect Classic treatment offers the best juice yields and has some impact on total concentration of VOCs with maximum dosage. Rohapect UF treatment offers largest increases in total concentration of VOCs but helps little with juice yields. Both are pectinases derived from a “classic” strain of *Aspergillus niger*. A mixture of these products might offer the best combined yields of juice and VOCs. The enzymes chosen for this study were all commercially available, except for the developmental enzyme NF10, and were known to be used in applications in food industry. The enzyme dosages were selected based on the recommended minimum and maximum dosages by the enzyme manufacturers.

The results propose that various VOCs can be impacted by the treatments used in this study, and that the use of enzymes during the processing of lingonberry juice has an impact on the volatile composition with different behaviours depending on the used treatment. Generally, lingonberries aroma is prevailed by terpenes, with alcohols, aldehydes, esters and ketones present in small quantities. VOCs with highest overall concentrations found were ethyl acetate, ethyl benzoate, 2-methyl-3-buten-2-ol, benzaldehyde, pentanal, hexanal, undecane, *α*-Pinene, linalool L and diacetyl.

The general observations Viljanen et al. (2014) made with enzymatic treatments were that the concentrations of aldehydes remained unchanged or decreased, the amount of 2-pentanone increased, and that the enzymes had little effect on terpenes, except for the amount of α-Pinene, which increased significantly. In this study the concentrations of aldehydes increased with all treatments, the concentration of 2-pentanone increased with all treatments except beta glucosidase 1-hour incubations, and terpene concentrations increased.

Continuing this study with sensory evaluation and GC-O would offer better understanding of the relation between measured volatiles and their impact on the sensory quality of enzymatically treated lingonberry juices. In order characterise

the changes in glycosidically bound volatiles during enzymatical treatments further research is required.

Terpenes were expected to be liberated from their glucoside precursors via the action of *β*-glucosidase during the enzyme treatment. This reaction did not occur in significance with these treatments with lingonberry juice. The answer might be that the terpene concentrations are just rather low in the studied lingonberries, and thus beta glucosidase treatment offers little use.

The assignment of key flavour compounds would be a logical next step in studying lingonberry VOCs. The contribution of compounds, such as these 34 identified in this study, to the overall aroma could be evaluated with the relative odour activity value (ROAV). This is a practise used by Multari et al. (2018) in University of Turku.

9 Acknowledgements

I thank M.Sc. (Tech) Niina Kelanne and Ph.D. Oskar Laaksonen for their guiding during this master's thesis work. I thank Ph.D. Alexis Marsol for his technical advice on GC-MS, peak integration, and retention indexes. Special thank you for my wife, Maisa.

References

Aaslyng, M.D., Elmore, J.S., Mottram, D.S., 1998. Comparison of the Aroma Characteristics of Acid-Hydrolyzed and Enzyme-Hydrolyzed Vegetable Proteins Produced from Soy. J. Agric. Food Chem. 46, 5225–5231. https://doi.org/10.1021/jf9806816

Acosta-Estrada, B.A., Gutiérrez-Uribe, J.A., Serna-Saldívar, S.O., 2014. Bound phenolics in foods, a review. Food Chem. 152, 46–55. https://doi.org/10.1016/j.foodchem.2013.11.093

Acree, T., Arn, H., 2004. Flavornet Home Page [WWW Document]. Flavornet Hum. Odor Space. URL http://flavornet.org/ (accessed 4.25.18).

Alejandro Vazquez-Cruz, M., Jimenez-Garcia, S.N., Torres-Pacheco, I., Horacio Guzman-Maldonado, S., Guevara-Gonzalez, R.G., Miranda-Lopez, R., 2012. Effect of Maturity Stage and Storage on Flavor Compounds and Sensory Description of Berrycactus (Myrtillocactus geometrizans). J. Food Sci. 77, C366–C373. https://doi.org/10.1111/j.1750-3841.2012.02621.x

Anjou, K., Sydow, E.V., 1967. The aroma of cranberries I. Vaccinium vitis-idaea L. Acta Chem. Scand. 21, 945–952.

Arthur, C.L., Pawliszyn, J., 1990. Solid phase microextraction with thermal desorption using fused silica optical fibers. Anal. Chem. 62, 2145–2148. https://doi.org/10.1021/ac00218a019

Aryan, A.., Wilson, B., Strauss, P.., 1987. The properties of glycosidases of Vitis vinifera and a comparison of their b-glucosidase activity with that of exogenous enzymes. An assessment of possible applications in enology. Am. J. Enol. Vitic. 182–188.

Babbar, N., Roy, S.V., Wijnants, M., Dejonghe, W., Caligiani, A., Sforza, S., Elst, K., 2016. Effect of Extraction Conditions on the Saccharide (Neutral and Acidic) Composition of the Crude Pectic Extract from Various Agro-Industrial Residues. J. Agric. Food Chem. 64, 268–276. https://doi.org/10.1021/acs.jafc.5b04394

Baek, H.H., Cadwallader, K.R., 1996. Volatile Compounds in Flavor Concentrates Produced from Crayfish-Processing Byproducts with and without Protease Treatment. J. Agric. Food Chem. 44, 3262–3267. https://doi.org/10.1021/jf960023q

Beaulieu, J.C., 2007. Quality Changes in Cantaloupe During Growth, Maturation, and in Stored Fresh-cut Cubes Prepared from Fruit Harvested at Various Maturities. J. Am. Soc. Hortic. Sci. 132, 720–728.

Brul, S., 1999. Preservative agents in foods Mode of action and microbial resistance mechanisms. Int. J. Food Microbiol. 50, 1–17. https://doi.org/10.1016/S0168-1605(99)00072-0

Bujor, O.-C., Ginies, C., Popa, V.I., Dufour, C., 2018. Phenolic compounds and antioxidant activity of lingonberry (Vaccinium vitis-idaea L.) leaf, stem and fruit at different harvest periods. Food Chem. 252, 356–365. https://doi.org/10.1016/j.foodchem.2018.01.052

Cabaroglu, T., Selli, S., Canbas, A., Lepoutre, J.-P., Günata, Z., 2003. Wine flavor enhancement through the use of exogenous fungal glycosidases. Enzyme Microb. Technol. 33, 581–587. https://doi.org/10.1016/S0141-0229(03)00179-0

Campbell, R.E., Drake, M.A., 2013. Invited review: The effect of native and nonnative enzymes on the flavor of dried dairy ingredients. J. Dairy Sci. 96, 4773–4783. https://doi.org/10.3168/jds.2013-6598

Campbell-Platt, G., International Union of Food Science and Technology (Eds.), 2009. Food science and technology. Wiley-Blackwell ; IUFoST, Chichester, West Sussex, U.K. ; Ames, Iowa : [Oakville, Ont.].

CBI, Ministry of Foreign Affairs, 2018. Exporting fresh berries to Europe [WWW Document]. URL https://www.cbi.eu/node/2074/pdf/

Ek, S., Kartimo, H., Mattila, S., Tolonen, A., 2006. Characterization of Phenolic Compounds from Lingonberry (*Vaccinium vitis-idaea*). J. Agric. Food Chem. 54, 9834–9842. https://doi.org/10.1021/jf0623687

Farneti, B., Khomenko, I., Grisenti, M., Ajelli, M., Betta, E., Algarra, A.A., Cappellin, L., Aprea, E., Gasperi, F., Biasioli, F., Giongo, L., 2017. Exploring Blueberry Aroma Complexity by Chromatographic and Direct-Injection Spectrometric Techniques. Front. Plant Sci. 8. https://doi.org/10.3389/fpls.2017.00617

Flutto, L., 2003. PECTIN | Properties and Determination, in: Encyclopedia of Food Sciences and Nutrition. Elsevier, pp. 4440–4449. https://doi.org/10.1016/B0-12-227055-X/00901-9

Grassin, C., Fauquembergue, P., 1996. Application of pectinases in beverages, in: Progress in Biotechnology. Elsevier, pp. 453–462. https://doi.org/10.1016/S0921-0423(96)80275-9

Gueguen, Y., Chemardin, P., Janbon, G., Arnaud, A., Galzy, P., 1996. A Very Efficient β-Glucosidase Catalyst for the Hydrolysis of Flavor Precursors of Wines and Fruit Juices. J. Agric. Food Chem. 44, 2336–2340. https://doi.org/10.1021/jf950360j

Hadi, M.E., Zhang, F.-J., Wu, F.-F., Zhou, C.-H., Tao, J., 2013. Advances in Fruit Aroma Volatile Research. Molecules 18, 8200–8229. https://doi.org/10.3390/molecules18078200

Hui, Y.H. (Ed.), 2010. Handbook of fruit and vegetable flavors. Wiley, Hoboken, N.J.

Iversen, C.K., Jakobsen, H.B., Olsen, C.-E., 1998. Aroma Changes during Black Currant (Ribes nigrum L.) Nectar Processing. J. Agric. Food Chem. 46, 1132–1136. https://doi.org/10.1021/jf970513y

Kafkas, E., Kafkas, S., 2005. Comparison of Methodologies for the Identification of Aroma Compounds in Strawberry. Turk. J. Agric. For. 29, 383–390.

Kalua, C.M., Allen, M.S., Bedgood, D.R., Bishop, A.G., Prenzler, P.D., Robards, K., 2007. Olive oil volatile compounds, flavour development and quality: A critical review. Food Chem. 100, 273–286. https://doi.org/10.1016/j.foodchem.2005.09.059

Kilcawley, K.N., Wilkinson, M.G., Fox, P.F., 1998. Enzyme-modified cheese. Int. Dairy J. 8, 1–10. https://doi.org/10.1016/S0958-6946(98)00010-7

Leino, M., Kallio, H., 1993. Volatile compounds of blackcurrant juice and wine. Z. F�r Lebensm.-Unters. -Forsch. 196, 410–414. https://doi.org/10.1007/BF01190803

Longo, M.., Sanromán, M.., 2006. Production of Food Aroma Compounds : Microbial and Enzymatic Methodologies. Food Technol. Biotechnol. 44, 335–353.

Maehashi, K., Matsuzaki, M., Yamamoto, Y., Udaka, S., 1999. Isolation of Peptides from an Enzymatic Hydrolysate of Food Proteins and

Characterization of Their Taste Properties. Biosci. Biotechnol. Biochem. 63, 555–559. https://doi.org/10.1271/bbb.63.555

Malowicki, S.M.M., Martin, R., Qian, M.C., 2008. Volatile Composition in Raspberry Cultivars Grown in the Pacific Northwest Determined by Stir Bar Sorptive Extraction−Gas Chromatography−Mass Spectrometry. J. Agric. Food Chem. 56, 4128–4133. https://doi.org/10.1021/jf073489p

Marsol-Vall, A., Kortesniemi, M., Karhu, S.T., Kallio, H., Yang, B., 2018. Profiles of Volatile Compounds in Blackcurrant (*Ribes nigrum*) Cultivars with a Special Focus on the Influence of Growth Latitude and Weather Conditions. J. Agric. Food Chem. 66, 7485–7495. https://doi.org/10.1021/acs.jafc.8b02070

Martínez-Anaya, M.A., 1996. Enzymes and Bread Flavor. J. Agric. Food Chem. 44, 2469–2480. https://doi.org/10.1021/jf960020d

Marušić, N., Petrović, M., Vidaček, S., Petrak, T., Medić, H., 2011. Characterization of traditional Istrian dry-cured ham by means of physical and chemical analyses and volatile compounds. Meat Sci. 88, 786–790. https://doi.org/10.1016/j.meatsci.2011.02.033

Miettinen, S.-M., 2004. Instrumentally measured release and human perception of aroma compounds from foods and model systems differing in fat content. University of Helsinki, Helsinki.

Mojsov, K.D., 2016. Aspergillus Enzymes for Food Industries, in: New and Future Developments in Microbial Biotechnology and Bioengineering. Elsevier, pp. 215–222. https://doi.org/10.1016/B978-0-444-63505-1.00033-6

Multari, S., Marsol-Vall, A., Yang, B., Suomela, J.-P., 2018. Effects of Aromatic Herb Flavoring on Carotenoids and Volatile Compounds in Edible Oil From Blue Sweet Lupin (*Lupinus angustifolius*). Eur. J. Lipid Sci. Technol. 120, 1800227. https://doi.org/10.1002/ejlt.201800227

Nadar, S.S., Rao, P., Rathod, V.K., 2018. Enzyme assisted extraction of biomolecules as an approach to novel extraction technology: A review. Food Res. Int. 108, 309–330. https://doi.org/10.1016/j.foodres.2018.03.006

Ni, H., Hao, S., Zheng, F., Zhang, L., Lee, B., Wang, Y., Chen, F., 2017. Effects of two enzyme extracts of Aspergillus niger on green tea aromas. Food Sci. Biotechnol. 26, 611–622. https://doi.org/10.1007/s10068-017-0108-0

Nič, M., Jirát, J., Košata, B., Jenkins, A., McNaught, A. (Eds.), 2009. retention indexin column chromatography, I, in: IUPAC Compendium of Chemical Terminology. IUPAC, Research Triagle Park, NC. https://doi.org/10.1351/goldbook.R05360

Nile, S.H., Park, S.W., 2014. Edible berries: Bioactive components and their effect on human health. Nutrition 30, 134–144. https://doi.org/10.1016/j.nut.2013.04.007

Pennerman, K.K., AL-Maliki, H.S., Lee, S., Bennett, J.W., 2016. Fungal Volatile Organic Compounds (VOCs) and the Genus Aspergillus, in: New and Future Developments in Microbial Biotechnology and Bioengineering. Elsevier, pp. 95–115. https://doi.org/10.1016/B978-0-444-63505-1.00007-5

Reineccius, G., 2010. Flavor of Small Fruits, in: Qian, M.C., Rimando, A.M. (Eds.), Flavor and Health Benefits of Small Fruits. American Chemical Society, Washington, DC, pp. 3–11. https://doi.org/10.1021/bk-2010-1035.ch001

Riu-Aumatell, M., López-Tamames, E., Buxaderas, S., 2005. Assessment of the Volatile Composition of Juices of Apricot, Peach, and Pear According to Two Pectolytic Treatments. J. Agric. Food Chem. 53, 7837–7843. https://doi.org/10.1021/jf051397z

Rizzolo, A., Polesello, A., Polesello, S., 1992. Use of headspace capillary GC to study the development of volatile compounds in fresh fruit. J. High Resolut. Chromatogr. 15, 472–477. https://doi.org/10.1002/jhrc.1240150713

Rocha, S.M., Coutinho, P., Delgadillo, I., Cardoso, A.D., Coimbra, M.A., 2005. Effect of enzymatic aroma release on the volatile compounds of white wines presenting different aroma potentials. J. Sci. Food Agric. 85, 199–205. https://doi.org/10.1002/jsfa.1937

Salas, N.A., Molina-Corral, F.J., González-Aguilar, G.A., Otero, A., Sepulveda, D.R., Olivas, G.I., 2011. Volatile production by 'Golden Delicious' apples is affected by preharvest application of aminoethoxyvinylglycine. Sci. Hortic. 130, 436–444. https://doi.org/10.1016/j.scienta.2011.07.017

Schieberle, P., Hofmann, T., 1997. Evaluation of the Character Impact Odorants in Fresh Strawberry Juice by Quantitative Measurements and Sensory Studies on Model Mixtures. J. Agric. Food Chem. 45, 227–232. https://doi.org/10.1021/jf960366o

Su, E., Xia, T., Gao, L., Dai, Q., Zhang, Z., 2010. Immobilization of β-glucosidase and its aroma-increasing effect on tea beverage. Food Bioprod. Process. 88, 83–89. https://doi.org/10.1016/j.fbp.2009.04.001

Sullivan, G.A., Calkins, C.R., 2010. Application of exogenous enzymes to beef muscle of high and low-connective tissue. Meat Sci. 85, 730–734. https://doi.org/10.1016/j.meatsci.2010.03.033

Sun, W.-X., Hu, K., Zhang, J.-X., Zhu, X.-L., Tao, Y.-S., 2018. Aroma modulation of Cabernet Gernischt dry red wine by optimal enzyme treatment strategy in winemaking. Food Chem. 245, 1248–1256. https://doi.org/10.1016/j.foodchem.2017.11.106

Tian, Y., Puganen, A., Alakomi, H.-L., Uusitupa, A., Saarela, M., Yang, B., 2018. Antioxidative and antibacterial activities of aqueous ethanol extracts of berries, leaves, and branches of berry plants. Food Res. Int. 106, 291–303. https://doi.org/10.1016/j.foodres.2017.12.071

Toldrá, F., Flores, M., 2000. The use of muscle enzymes as predictors of pork meat quality. Food Chem. 69, 387–395. https://doi.org/10.1016/S0308-8146(00)00052-2

Turtiainen, M., Salo, K., Saastamoinen, O., 2011. Variations of Yield and Utilisation of Bilberries (Vaccinium myrtillus L.) and Cowberries (V. vitis-idaea L.) in Finland. Silva Fenn. 45, 237–251.

Vaara, M., 2015. Luonnonmarjojen käyttö kotitalouksissa ja teollisuudessa, in: Metsä. Monikäyttö Ja Ekosysteemipalvelut. Luonnonvarakeskus (Luke), Helsinki.

Verbeke, W., 2006. Functional foods: Consumer willingness to compromise on taste for health? Food Qual. Prefer. 17, 126–131. https://doi.org/10.1016/j.foodqual.2005.03.003

Viljakainen, S., Visti, A., Laakso, S., 2002. Concentrations of Organic Acids and Soluble Sugars in Juices from Nordic Berries. Acta Agric. Scand. Sect. B — Soil Plant Sci. 52, 101–109. https://doi.org/10.1080/090647102321089846

Viljanen, K., Heiniö, R.-L., Juvonen, R., Kössö, T., Puupponen-Pimiä, R., 2014. Relation of sensory perception with chemical composition of bioprocessed lingonberry. Food Chem. 157, 148–156. https://doi.org/10.1016/j.foodchem.2014.02.030

Visti, A., Viljakainen, S., Laakso, S., 2003. Preparation of fermentable lingonberry juice through removal of benzoic acid by Saccharomyces cerevisiae yeast. Food Res. Int. 36, 597–602. https://doi.org/10.1016/S0963-9969(03)00007-3

Wang, B., Xu, S., 2009. Effects of different commercial lipases on the volatile profile of lipolysed milk fat. Flavour Fragr. J. 24, 335–340. https://doi.org/10.1002/ffj.1945

Whitehurst, R.J., Oort, M. van (Eds.), 2010. Enzymes in food technology, 2nd ed. ed. Wiley-Blackwell, Chichester, U.K. ; Ames, Iowa.

Worthington Biochemical Corporation, 1972. Manual of Clinical Enzyme Measurements.

Zhang, S.B., Wang, Z., Xu, S.Y., 2007. Optimization of the Aqueous Enzymatic Extraction of Rapeseed Oil and Protein Hydrolysates. J. Am. Oil Chem. Soc. 84, 97–105. https://doi.org/10.1007/s11746-006-1004-6

Zhang, X.-B., Du, X.-F., 2015. Effects of Exogenous Enzymatic Treatment During Processing on the Sensory Quality of Anxi Tieguanyin Summer Oolong Tea. Food Technol. Biotechnol. 53. https://doi.org/10.17113/ftb.53.02.15.3642

Zhu, J., Chen, F., Wang, L., Niu, Y., Chen, H., Wang, H., Xiao, Z., 2016. Characterization of the Key Aroma Volatile Compounds in Cranberry (*Vaccinium macrocarpon* Ait.) Using Gas Chromatography–Olfactometry (GC-O) and Odor Activity Value (OAV). J. Agric. Food Chem. 64, 4990–4999. https://doi.org/10.1021/acs.jafc.6b01150