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Phytochemical Analysis and Bioactivity Screening of Vaccinium uliginosum L. Fruits

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ABSTRACT

Vaccinium uliginosum (bog bilberry) is commonly consumed in North America and parts of Asia, whereas its use has traditionally been avoided in several European regions due to its alleged toxicity. However, the scientific evidence supporting these claims remains limited. Previous work within this project called the "Bog Bilberry Enigma" have mainly focused on investigating the apolar berry constituents.

The aim of this master's thesis was to investigate the phytochemical composition, potential toxicity and biological activity of *V. uliginosum* fruits, with a focus on samples from different geographical origins. Aditionally, the possible influence of *Monilinia megalospora* infection and fermentation processes on berry composition and bioactivity were examined.

Berry samples were analysed using ultra-high-performance liquid chromatography (UHPLC) and ultra-high-performance supercritical fluid chromatography (UHPSFC). Biological activity and potential toxicity were evaluated using in vitro assays with GFP-tagged human liver cells (Huh-7) and in vivo experiments with *Caenorhabditis elegans*. Fermentation experiments were conducted to assess the fermentation capacity of bog bilberries under different circumstances, as well as in comparison with *Vaccinium myrtillus* and *Vaccinium corymbosum* fruits.

Overall, the findings suggest that bog bilberries are unlikely to be inherently toxic. The results revealed only minor differences in metabolite patterns between samples from different regions and provided no evidence for the presence of toxic constituents. However, fermentation experiments demonstrated a notable ethanol production capacity of the bog bilberries, providing a plausible explanation for historical reports describing intoxicating and inebriating effects recorded after berry consumption.

ZUSAMMENFASSUNG

Vaccinium uliginosum (Rauschbeere) wird in Nordamerika und Teilen Asiens seit langem als Nahrungs- und Heilmittel genutzt, während der Verzehr in mehreren europäischen Regionen aufgrund vermeintlich toxischer Wirkungen traditionell gemieden wird. Eine fundierte wissenschaftliche Grundlage fehlt bislang, weshalb das Projekt „Bog Bilberry Enigma“ ins Leben gerufen wurde, bei dem bisher vor allem die apolaren Inhaltsstoffe der Beeren untersucht wurden.

Ziel der Masterarbeit war es, die phytochemische Zusammensetzung sowie die biologische Aktivität und das potenziell toxische Profil der Rauschbeerfrüchte systemisch zu erfassen. Dabei wurden Proben unterschiedlicher geografischer Herkunft verglichen und der mögliche Einfluss einer Infektion mit *Monilina megalospora* sowie von Fermentationsprozessen auf die chemische Zusammensetzung und Bioaktivität der Beeren untersucht.

Die Analyse erfolgte mittels ultra-high-performance liquid chromatography (UHPLC) und ultra-high-performance supercritical fluid chromatography (UHPSFC). Zur Bewertung der biologischen Aktivität und Toxizität wurden sowohl in-vitro Untersuchungen an GFP-markierten humanen Leberzellen (Huh-7) als auch in-vivo Experimente am Modellorganismus *Caenorhabditis elegans* durchgeführt. Ergänzend wurde im Rahmen von Fermentationsexperimenten die Ethanolbildung im Rauschbeersaft unter verschiedenen Bedingungen sowie im Vergleich zu verwandten *Vaccinium*-Arten untersucht.

Die Ergebnisse zeigen, dass Rauschbeeren nicht als toxisch einzustufen sind. Unterschiede in den Metabolitenprofilen waren gering und toxische Effekte konnten nicht beobachtet werden. Die ausgeprägte Fermentationsfähigkeit der Rauschbeere liefert jedoch eine plausible Erklärung für historische Berichte über die berauschende Wirkung nach dem Verzehr.

LIST OF ABBREVIATIONS

ACN	Acetonitrile
ABV	Alcohol content by volume
BEH	Bridged ethylene hybrid
CAT	Catechin
$CHCl_3$	Chloroform
CRP	C-reactive protein
ddH_2O	double-distilled water
DMSO	Dimethylsulfoxide
ELSD	Evaporative light scattering detector
eNOS	Endothelial nitric oxide
EtAc	Ethyl acetate
FA	Formic acid
FU	Fluorouracil
H2O	Water
H2SO4	Sulfuric acid
LLE	Lead-like enhanced
MCP-1	Monocyte chemotactic protein
MeOH	Methanol
m/z	Mass to charge ratio
NO	Nitric oxide
PDA	Photo diode array
QDa	Quadrupole dalton
Rpm	Revolutions per minute
TLC	Thin layer chromatography
TMA	Total monomeric anthocyanins
TSP	Total soluble polyphenols
UHPLC	Ultra-high-performance liquid chromatography
UHPSFC	ultra-high-performance supercritical fluid chromatography
UA	Ursolic acid
UV	Ultraviolet

1 INTRODUCTION

1.1 Botanical and Historical Background of *Vaccinium uliginosum*

The bog bilberry (*Vaccinium uliginosum* L.), belonging to the Ericaceae family, is a species that thrives in acidic, peaty soils, but has proven to be ecologically adaptable to a wide range of environments. It is widely distributed across boreal and arctic regions. In lower latitudes, particularly in Central Europe and Asia, the bog bilberry is regarded as a glacial relict, restricted to high-altitude habitats. (Jacquemart, 1996)

Morphologically, *V. uliginosum* is a small deciduous shrub that grows up to 15 – 70 cm in height. It bears brownish twigs with obovate to lanceolate blue-green leaves, with entire margins (see Figure 1). The plants flowering period lasts from May to June, displaying approximately 5 mm long urn-shaped corollas with white to pale pink petals. In the months of August and September the berries start to ripen, typically spread-out solitary or in small clusters. The round fruits, about 10 mm in diameter, display a dusty blue peel on the surface and contain a greenish-white pulp with various small seeds. (Jacquemart, 1996; *'POWO*, 2025)

Figure 1: Bog bilberry, location: Klin, Slovakia, photo made by Zuzana Vaneková

Despite the wide distribution of the bog bilberry, cultural perceptions vary considerably between the regions. These different attitudes toward the berry are also reflected in the local vernacular names. For example, in northern and eastern Europe avoidance of the fruit became common in some regions, as there are several reports from the early 20th century which have associated its consumption with

dizziness, nausea, headaches or even hallucinatory effects. This explains common names such as the German *Rauschbeere* (“inebriating berry”), Slovak *Šialenica* ("mad berry”), Czech *Blinkavka* (“vomit berry”) and Lithuanian *girtuoklė* (“drunken berry”). By contrast, in North America the bog bilberry has traditionally been consumed by indigenous tribes both as food source and as a medicinal plant. Different parts of the plant, including the leaves and berries, were used to prepare teas for the treatment of colds and coughs, topical applications for skin diseases, as well as remedies for nausea and diarrhoea. Similarly, in Russia and Siberia *V. uliginosum* is widely used as a food source by indigenous populations such as the Yakuts/Sakha, Nenets and Kamchadal, as well as by the non-indigenous population of Siberia. While no toxic effects are reported, the plant is described as part of shamanistic practices of some Siberian tribes. However, consequences of intoxication are exclusively linked to the alcohol content of fermented products rather than the berry itself. In the alpine and boreal parts of China and Mongolia, where the species is also widespread, it is also regarded as edible and incorporated into traditional preparations (Vaneková et al., 2024).

These cross-cultural differences highlight the unique dual reputation of *V. uliginosum.* Several explanations for this discrepancy have been proposed, including misidentifications with morphologically similar plants such as deadly nightshade (*Atropa belladonna* L.), *Paris quadrifolia* L., *Andromeda polifolia* L. or *Rhododendron tomentosum*, (syn. *Ledum palustre* L.). Other plausible factors include the accidental consumption of spoiled or fungus-infected berries, as well as individual intolerances or allergic reactions. Another explanation is that the associations with inebriation may have arisen from the fermented products made from the berries rather than from the fresh fruits themselves. Furthermore, the lack of studies documenting the phytochemical compositions of the berries from different regions leaves open the possibility of chemical variation, such as the presence of alkaloids, although this remains hypothetical. If such intoxicating constituents were present, they would most likely be expected in European berry samples, where negative reports have been most frequent, while their absence in North American bog bilberry samples would be consistent with the long history of safe use (Vaneková et al., 2024). Possible reasons for the alleged toxicity are summarized in Figure 2.

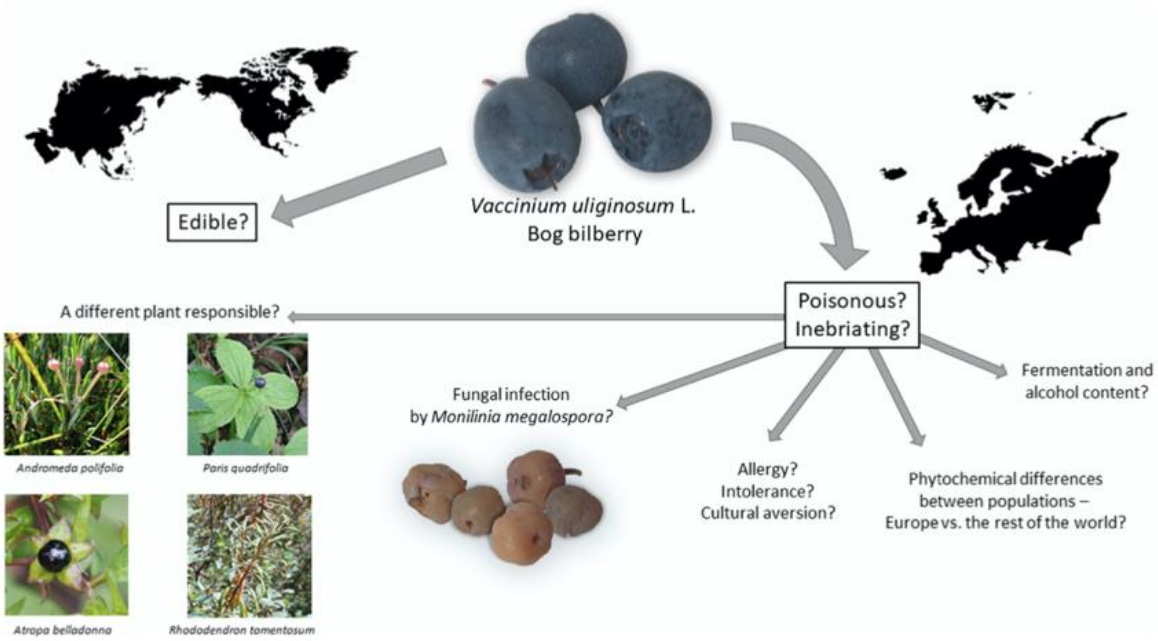

Figure 2: Possible reasons for the toxicity of bog bilberry, Image adapted from Vaneková, Holloway and Rollinger, 2024

1.2 *V. uliginosum* Composition

Beyond its cultural and historical significance, the bog bilberry has become a subject of scientific interest due to its complex phytochemical composition. While it is especially rich in bioactive compounds such as anthocyanins and flavanols, the abundance of these compounds can fluctuate with environmental influences, e.g. differences in altitude (Su et al., 2016a). A brief overview of its general phytochemical composition is provided in the following chapters.

1.2.1 General Characteristics & Basic Components

The pH value of bog bilberries is relatively high (pH = 3.5) in comparison to other berry species and their titratable acidity is moderate, measured at 1 g of citric acid per 100 g (Colak et al., 2016). The predominate soluble sugar is fructose, followed by glucose, which are generally the main sugars in several *Vaccinium* species such as highbush blueberries (*V. corymbosum*), lowbush blueberries (*V. angustifolium*) or bilberries (*V. myrtillus*). Colak et al. (2016) also reported that sucrose was not detectable in bog bilberries, a finding consistent with the review by Nestby et al. (2010), which likewise noted the absence or very low levels of measurable sucrose in the before mentioned *Vaccinium* species.

1.2.2 Known Constituents

Flavonols

Like mentioned before, bog bilberry fruits are rich in anthocyanins and flavonols and their characteristic profile of these compounds provide a distinctive phytochemical profile that could be used to distinguish *V. uliginosum* from *V. myrtillus* (Lätti et al., 2010). The identified flavonols include kaempferol, isorhamnetin, laricitrin, syringetin, myricetin and quercetin, occurring mainly as hexosides, pentosides and glucoronides. Quercetin and myricetin also appear in arabinoside forms. The bog bilberry juice is particularly rich in myricetin-3-O-galactoside and quercetin-3-O-galactoside. Among the flavanols, epicatechin and epigallocatechin have been identified as major constituents (Kopystecka et al., 2023; Wei et al., 2018).

Anthocyanins

The anthocyanins in *V. uliginosum* consist of five principal aglycones, being delphinidin, malvidin, cyanidin, peonidin and petunidin, which are connected to sugars such as arabinose, glucose, xylose or galactose (Lätti et al., 2010). According to Wei (2018), malvidin derivates account for approximately half of the total anthocyanin content. The remaining anthocyanins can be ranked based on their occurrence as follows: peonidin < cyanidin = petunidin < delphinidin.

Proanthocyanidins

The proanthocyanidin profile of the bog bilberry fruits is dominated by the monomer epicatechin and the dimer procyanidin B2, accompanied by moderate levels of phlorizin and taxifolin. In addition, comparatively high concentrations of gallocatechin and epigallocatechin have been reported, which stands in contrast to other *Vaccinium* species where these compounds are generally only found in trace amounts (Wang et al., 2021). Tannins, such as catechin together with epicatechin, have also been described in bog bilberry fruits (Ancillotti et al., 2016).

Acids

The most abundant phenolic acids detected in *V. uliginosum* were found to be protocatechuic and chlorogenic acid (Wei et al., 2018). Other studies have additionally identified caffeic, syringic, gallic, ferulic, salicylic and p-coumaric acid among the predominant phenolic acids of the bog bilberry (Ancillotti et al., 2016; Colak et al., 2016). Further compounds reported include the two species-specific benzoyloxyphenyl acetic acid derivatives vacciuligin A and B (Masuoka et al., 2007). As for organic acids, the most abundant ones in terms of concentration include citric, malic and ascorbic acids (Colak et al., 2016).

Coumarins and iridoids

Simple coumarins such as esculetin and scopoletin have been detected in bog bilberry fruits (Ancillotti et al., 2016) as well as iridoids including deacetylasperulosidic acid, loganic acid, monotropein, scandoside and splendoside (Choi et al., 2019; Heffels et al., 2017).

Wax composition

The surface of *V. uliginosum* berries is covered by a cuticular wax layer, more than half of which is composed of fatty acids, with arachidic acid as the principal component. In addition, the bog bilberry wax contains the triterpene acids oleanolic and ursolic acid. Further constituents include ketones, which represent the second largest fraction of the wax, along with minor amounts of aldehydes and alkanes (Trivedi et al., 2019a).

1.3 Effects of the Substance Groups

Bog bilberry fruits are highly valued for their rich content of biologically active secondary metabolites and as mentioned before, have been utilized in folk medicine for their health-promoting effects. The antioxidant, anti-inflammatory, antimicrobial, anticancer and neuroprotective properties of the berry are mainly attributed to its phenolic compounds. They can be generally divided into three groups of active compounds: flavonols, anthocyanins and phenolic acids (Colak et al., 2016; Kopystecka et al., 2023; Przybylski et al., 2025). The following section will go further into detail about their effects as well as potential areas of application.

Flavonols

Flavonols are primarily known for their antioxidant properties, as they act as radical scavengers preventing cardiovascular diseases and cancer development. Quercetin, one of the most abundant flavonols in *V. uliginosum*, exhibits cardioprotective effects through an enhancement of endothelial function by increasing endogenous NO (nitrite, nitrate and S-nitroso thiols) and reducing the endothelin-1 production, as well as a reduction of vascular remodeling and cardiac hypertrophy (Mahmud et al., 2023). Research has also linked quercetin with tyrosinase inhibitory activities. Tyrosinase is an important enzyme involved in melanin synthesis, therefore proposing potential use for dermal applications (Kim et al., 2009). Moreover, the possibility of using flavonols in cancer therapy is also linked to their ability to promote the death of cancer cells through the regulation of diverse forms of regulated cell death, including apoptosis, autophagy and necroptosis (Wendlocha et al., 2024).

Anthocyanins

Anthocyanins, the glycosylated derivatives of anthocyanidins, are pigments responsible for the blue and purple coloration of the berries (Khoo et al., 2017). Beyond their visual role, anthocyanins are especially known for their protective action in cardiovascular diseases mainly due to their antioxidant and anti-inflammatory activity. They enhance endothelial function by increasing the production of nitric oxide (NO) through endothelial nitric oxide synthase (eNOS) upregulation, thereby enhancing vasodilation and vascular health. Furthermore, anthocyanins inhibit activation of the transcription factor NF-κB (involved in inflammation regulation), downregulating the production of pro-inflammatory mediators such as monocyte chemotactic protein-1 (MCP-1). Anthocyanins also help regulate cardiovascular risk factors by reducing concentrations of C-reactive protein (CRP) and preventing platelet aggregation (Wallace, 2011; Wang et al., 2006).

Phenolic acids

Phenolic acids also exhibit various biologically beneficial effects like antioxidant, anti-inflammatory and antimicrobial effects. Additionally, certain acids, such as hydroxy- and dihydroxyphenyl derivatives, have shown neuroprotective effects against oxidative and nitrosative stress–induced cell death in human neuroblastoma (SH-SY5Y) cells (Kiriyama et al., 2024). Studies have demonstrated that phenolic acids exhibit strong antimicrobial activity, effectively targeting many common foodborne pathogens and therefore pose an opportunity as potential food preservatives (Tian & Yang, 2023). The most frequently investigated compounds include caffeic, chlorogenic, ferulic and gallic acids. According to Liu (2025), their antimicrobial effects could be resulting from the disruption of bacterial cell membranes, interference with nucleic acids and alterations of important metabolic processes.

1.4 Aim of the Thesis

The aim of this master thesis is to systematically investigate the phytochemical composition, biological activity and the potential toxicity of *Vaccinium uliginosum* fruits (bog bilberries), to clarify their alleged poisonous or intoxicating properties. The work is part of the “Bog Bilberry Enigma” project, where previous studies have mainly focused on apolar substances of the berries (Rechberger, 2023; Rose, 2025). To address my research question, a combined approach incorporating analytical chemistry, bioactivity assays and fermentation studies was employed, combining ethnopharmacological and phytochemical methods (see Figure 3).

The chemical composition of the *V. uliginosum* samples collected in different geographical locations was examined using ultra-high-performance supercritical liquid chromatography (UHPLC) and ultra-

high-performance supercritical fluid chromatography (UHPSFC). The aim was to identify potential differences in metabolite patterns between samples from Europe and North America. The biological activity and potential toxicity of the extracts were evaluated using bioassays, both in vitro with GFp-tagged human liver cells (Huh-7) and in vivo with the model organism *Caenorhabditis elegans*. These experiments were designed to determine whether treatment with the extracts could affect organism lifespan or cell viability. Furthermore, the potential influence of *Monilina megalospora* fungal infection on toxicity was investigated, as was the potential alteration of the biochemical composition of compounds such as flavonoids and anthocyanidins by the fungus. Lastly, fermentation experiments were conducted to examine the natural fermentation ability of bog bilberries in comparison to two related *Vaccinium* species, *V. myrtillus* and *V. corymbosum*. The main objective in this experiment is to determine what alcohol concentrations can be achieved with or without the addition of sugar or yeast and whether these results might be able to explain the historical reports of intoxication or inebriation associated with the consumption of the berries.

Overall, this work seeks to provide a scientific foundation for the assessment of the phytochemical potential, bioactivity and the safety of *Vaccinium uliginosum,* thereby contributing to a better understanding of its traditional use and more importantly, the misconceptions surrounding its alleged toxicity.

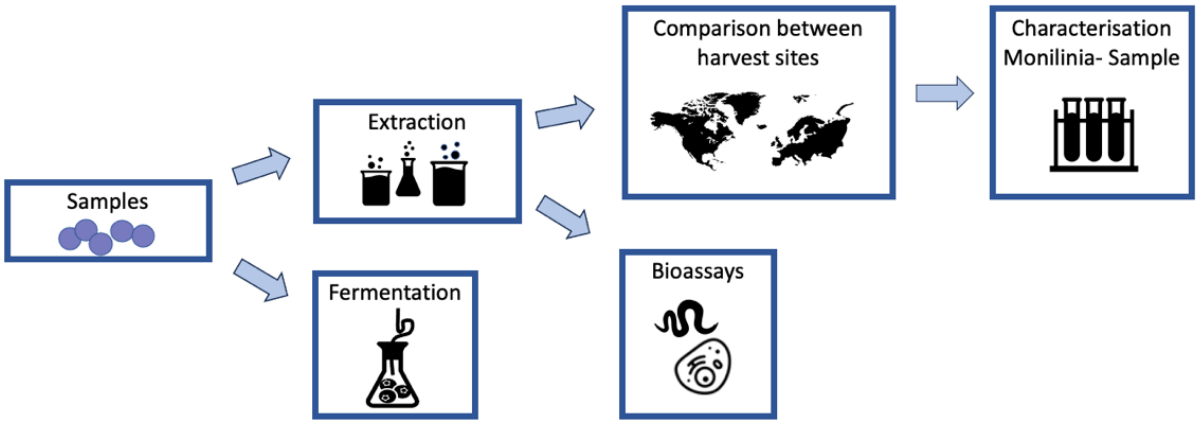

Figure 3: Workflow

2 RESULTS AND DISCUSSION

The origin and preparation of all samples are described in detail in the Materials and Methods section 4.1. For clarity, the following Table 1 provides an overview of the sample abbreviations used throughout the Results and Discussion section.

Table 1: samples utilized in this work, provided by Zuzana Vaneková

Abbreviation	Sample/Origin	comments
AB	*Atropa belladonna,* Austria	
AK2	*V. uliginosum,* Alaska	
AK2M	*V. uliginosum,* Alaska	M= *Monilinia megalospora* infected, testing for the fungus-infection hypothesis
AT3	*V. uliginosum,* Austria	
AT5	*V. uliginosum,* Austria	
CZ1	*V. uliginosum,* Czech Republic	
EE1	*V. uliginosum,* Estonia	
LP[1]	*Ledum palustre*	Testing for cross-contamination hypothesis
NO1	*V. uliginosum,* Norway	
SK2	*V. uliginosum,* Slovakia	
VM1	*V. myrtillus*	Comparison with different *Vaccinium* species

[1]To evaluate the hypothesis that toxic compounds from *Ledum palustre* (syn. *Rhododendron tomentosum*), which often grows near *V. uliginosum*, might adhere to the berry surface and contribute to its suspected toxicity, a reference sample of *L. palustre* was analysed. However, no matching compounds were detected in either the LLE extracts or the TLC analysis. Consequently, the sample was not investigated any further within the scope of this master's thesis.

2.1 Phytochemical Composition of *V. uliginosum*

2.1.1 TLC of Alkaloid Extracts

Using the extracts from samples AT3, AT5, CZ1, SK2, NO1, EE1, AK2, VM1, LP and AB using the Stas-Otto alkaloid extractions, thin layer chromatography (TLC) was performed as described in chapter 4.3.2 to get first insights into the phytochemical compositions. Atropine (A) from the deadly nightshade (*Atropa belladonna L*.) and common bilberry fruits (*V. myrtillus,* VM1) were used as control and reference samples for berries with and without alkaloids, respectively. Ursolic acid (UA) served as a reference for triterpene acids contents.

Dragendorff reagent specifically indicates the presence of alkaloids, producing characteristic orange to brown spots on TLC plates. As shown in Figure 4 and Figure 5, alkaloids were clearly detected in reference A as well as the sample AB. Using the chloroform-acetone (1 : 1) mobile phase, the alkaloid spots appeared at the baseline (Rf value = 0), which is consistent with the high polarity of these compounds. In Figure 5, when toluene-ethyl acetate-diethylamine (70:20:10) was used as the mobile phase, the alkaloid bands of AB and A were observed at Rf values of approximately 0.2 and 0.4. However, no Dragendorff-positive bands were detected in any of the bog bilberry extracts, indicating the absence of detectable alkaloids under the applied conditions.

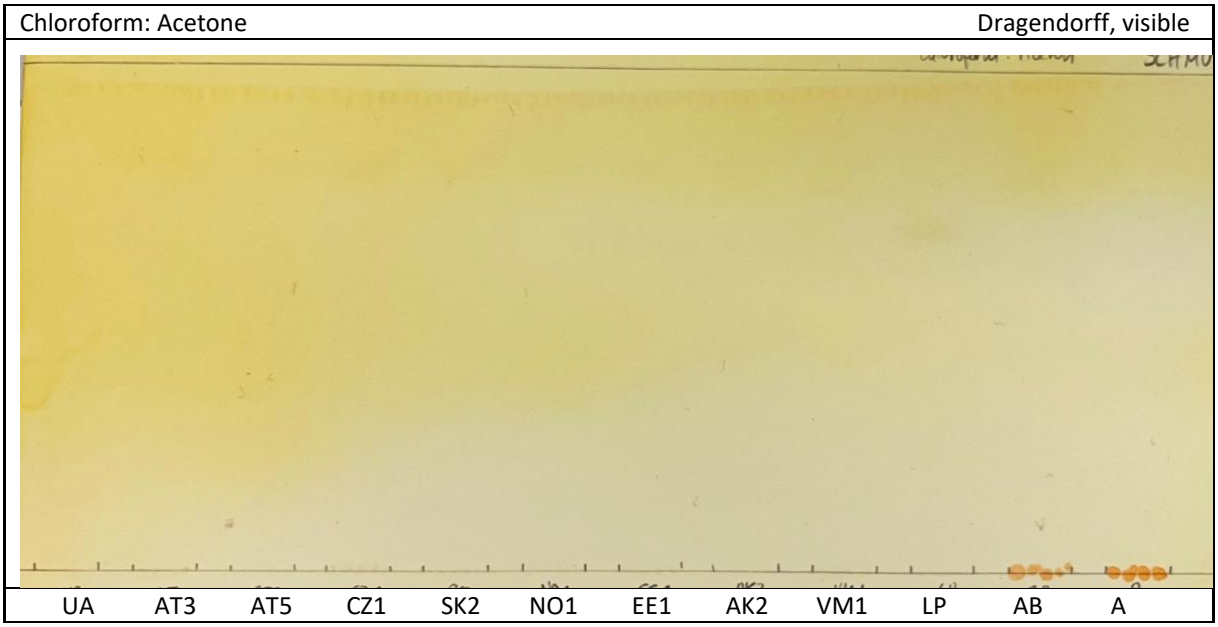

Figure 4: TLC plate (mobile phase Chloroform and Acetone, derivatization with Dragendorff reagent)

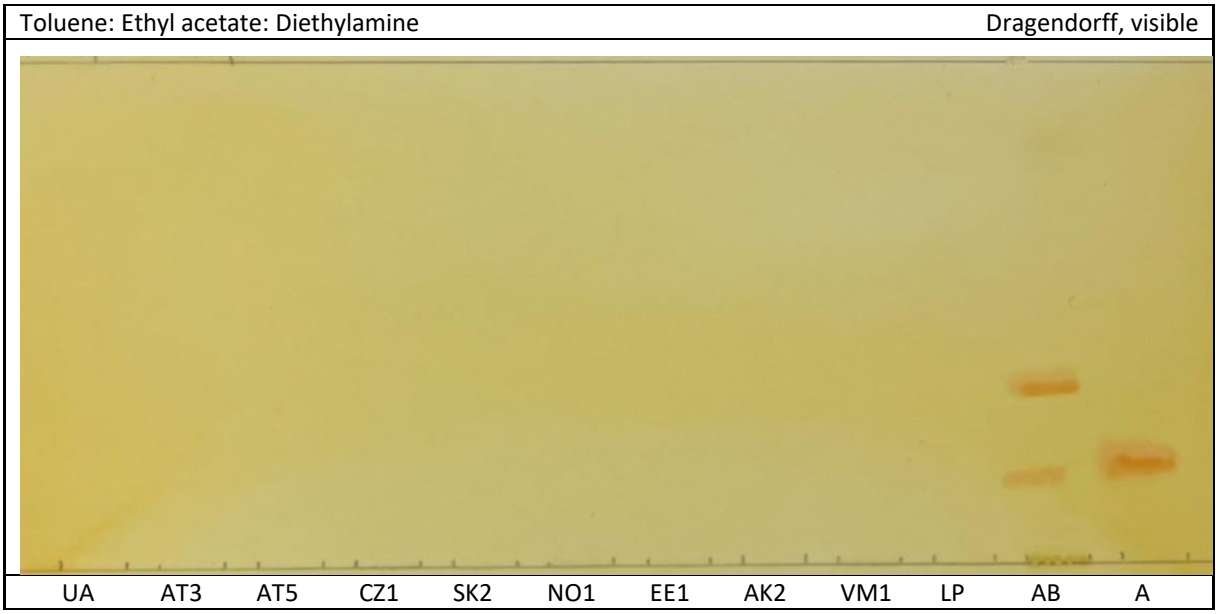

Figure 5: TLC plate (mobile phase Toluene, Ethyl acetate and Diethylamine, derivatization with Dragendorff reagent)

Vanillin-sulfuric acid derivatization was carried out as a complementary approach to Dragendorff staining. The chromatograms depicted in Figure 6 and Figure 7 confirm the presence of phytosterols at an Rf value of approximately 0.8, appearing in a characteristic blue-violet coloration when analysed under UV 254 nm after derivatization. In addition, yellowish bands at around Rf 0.7 are indicating triterpene acids, which is also in correspondence to the band of reference substance UA. Ursolic and oleanolic acids are reported as the most abundant triterpene acids in bog bilberries (Trivedi et al., 2019b).

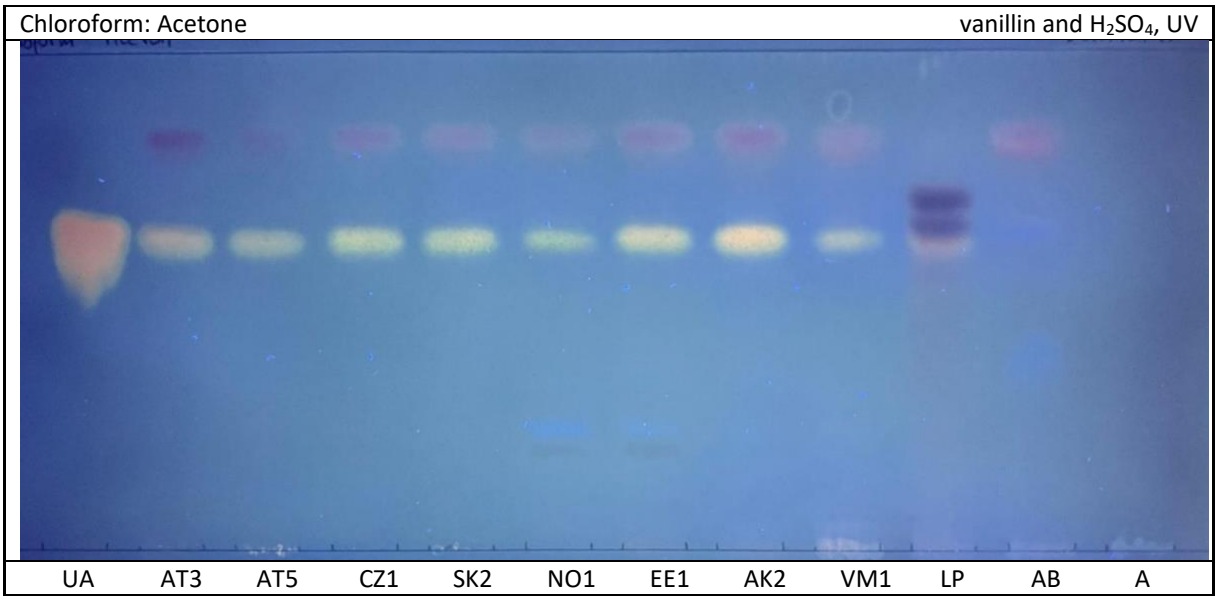

Figure 6: TLC plate (mobile phase Chloroform and Acetone, derivatization with vanillin and H_2SO_4)

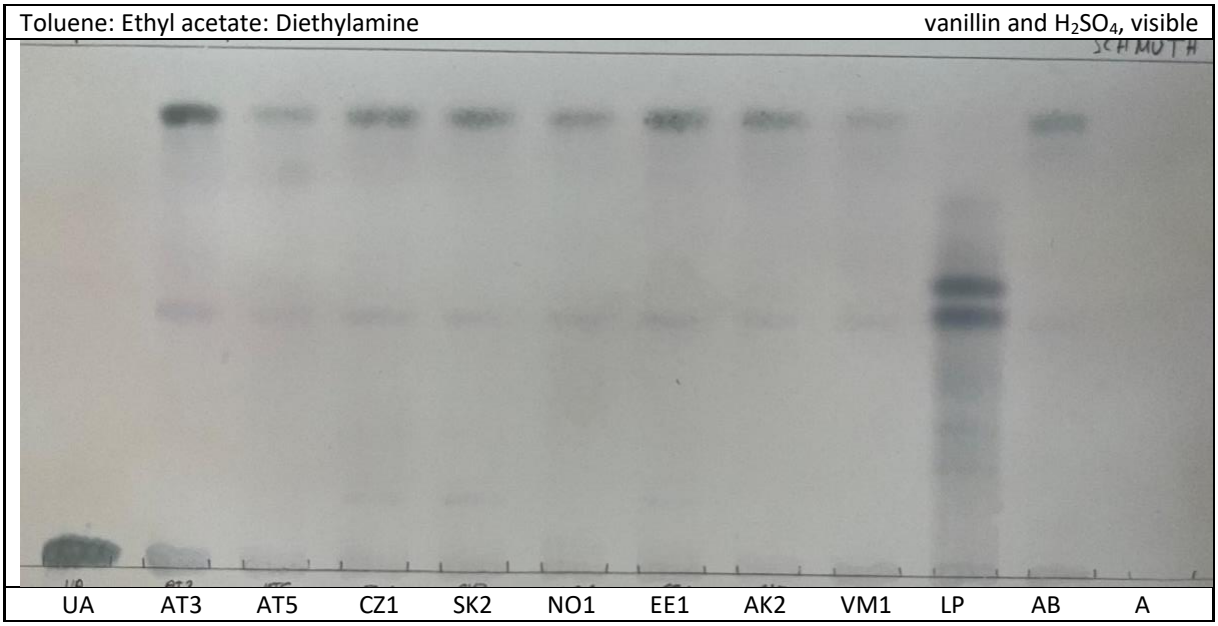

Figure 7: TLC plate (mobile phase Toluene, Ethyl acetate and Diethylamine, derivatization with vanillin and H_2SO_4)

2.1.2 Total Soluble Polyphenols and Total Monomeric Anthocyanins

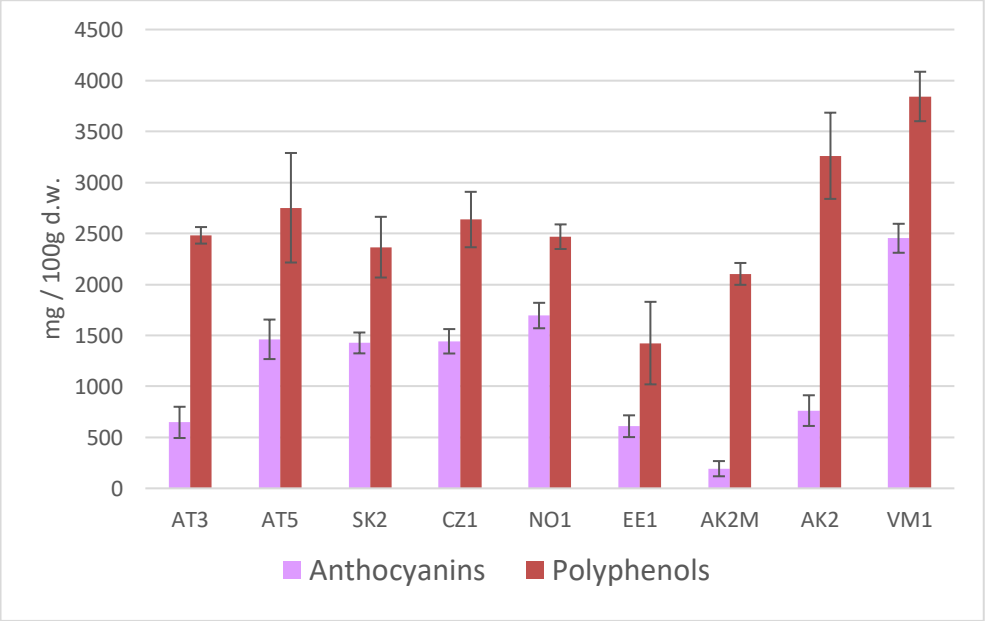

Figure 8: TMA and TSP of all LLE extracts

The bar chart in Figure 8 illustrates the amount of TSP and total monomeric anthocyanins (TMA) found in seven different *V. uliginosum* samples compared with a *V. myrtillus* (bilberry) sample. The total soluble polyphenol (TSP) content of the extracts was calculated according to the (+)-catechin calibration curve mentioned in chapter 4.3.5. The polyphenol content was higher in the bilberry sample

(VM1 3843.57 mg CAT/100 g d.w.) compared to the bog bilberry samples. This finding aligns with results by previous studies and can be explained by the presence of anthocyanins in the pulp as well as the peel (Ancillotti et al., 2016). Moreover, some variation regarding the TSP amount among the bog bilberry samples was observed, with the lowest amount being 1434.59 mg CAT/100 g d.w. in EE1 vs. the highest amount being 3261.78 mg CAT/100 g d.w. in AK2, which suggests possible influences from environmental factors and different growing conditions due to their separate locations. Factors such as temperature, light exposure, soil composition and water availability can influence the plant's production of secondary metabolites. Berries from colder or in general more harsher growing conditions often contain higher levels of metabolites like polyphenols (Lätti et al., 2010; Wang et al., 2014). Furthermore, the stage of ripeness at which the berries are picked can have an influence on their phytochemical contents (Kopystecka et al., 2023).

The TMA content of the extracts was calculated according to the CYA-3-GLU calibration curve mentioned in chapter 4.3.5. The results are presented in Figure 8. The outcome for total anthocyanin content showed a similar trend to the TSP contents. The bilberry (*V. myrtillus*) sample had an overall higher TMA value (VM1 2452.62 mg TAM/100 g d.w.) compared to the bog bilberry (*V. uliginosum*) samples. Among the bog bilberry samples, the TMA content varied significantly, ranging from 609.40 mg TMA/100 g d.w. in EE1 to 1694.76 mg TMA/100 g d.w. in AT5.

2.1.3 Individual Anthocyanins

The UPLC chromatogram PDA-spectrum at wavelength 530 nm (depicted in Figure 11 and Figure 12) shows the anthocyanin content determined in *V. uliginosum* and *V. myrtillus* (VM). The chromatographic profile shows a distinctive elution order of anthocyanins glycosylated with the same sugar across all samples: delphinidin (DEL) < cyanidin (CYA) < petunidin (PET) < peonidin (PEO) < malvidin (MAL). Anthocyanin identification was achieved by dereplicating the characteristic [M] + fragments in positive ion mode, followed by verification against reference compounds. This order aligns with the chemical properties of anthocyanins, as increased hydroxylation makes the compound elude faster, while structures with increased methylation decrease the polarity and therefore elude later. For example, delphinidin (Figure 9), with 3 OH-groups, is eluted before malvidin (Figure 10) because of its methylated structures. Additionally, for each anthocyanin the different glycosylation variants were eluted following the sequence: galactose (Gal) < glucose (Glc) < arabinose (Ara).

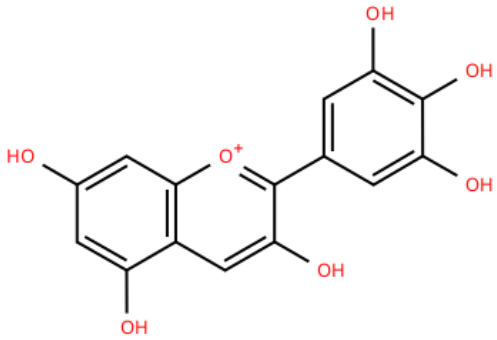

Figure 9: Delphinidin

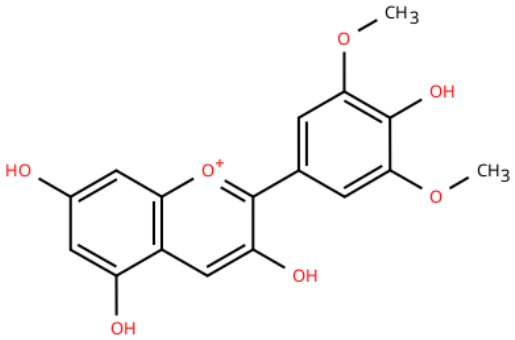

Figure 10: Malvidin

Whereas the same elution pattern and retention times were observed in both *Vaccinium* species, they differ regarding the amount of each anthocyanin contained. In the bog bilberry samples malvidin stands out as the most abundant anthocyanin, while the bilberry is particularly rich in cyanidin and delphinidin. Moreover, the chromatogram of the sample AK2 displayed notable differences compared to the other bog bilberry samples. Within the retention time of minute 12 to 14, the following anthocyanins were detected: delphinidin-glucose (del-glc) eluting first, followed by a double signal identified with cyanidin-galactose (cya-gal) and delphinidin-arabinose (del-ara). In the chromatogram of the AK2 sample, the content of del-glc was approximately 2 - 3 times higher than the combined content of cya-gal/del-ara. In contrast, the other bog bilberry samples exhibited comparable contents for these anthocyanins (del-glc, cya-gal and del-ara) in the same region. A similar difference in content was also observed for the following peaks: the alaskan sample contains proportionally more cyanidin-glucose and less cyanidin-arabinose/petunidin-galactose. In addition to that, only a low amount of peonidin-galactose and petunidin-arabinose was detected, as well as only trace amounts of malvidin-arabinose. AK2 was collected in Alaska, making it the only non-European sample in the study. This geographical distinction may account for the differences observed in metabolite pattern and intensities. Overall, the results observed in this study are comparable to previously published data for *V. uliginosum* from Italy, Turkey, Lithuania, Finland and China (Ancillotti et al., 2016; Colak et al., 2016; Kraujalytė et al., 2015; Lätti et al., 2010; Määttä-Riihinen et al., 2004; Su et al., 2016b).

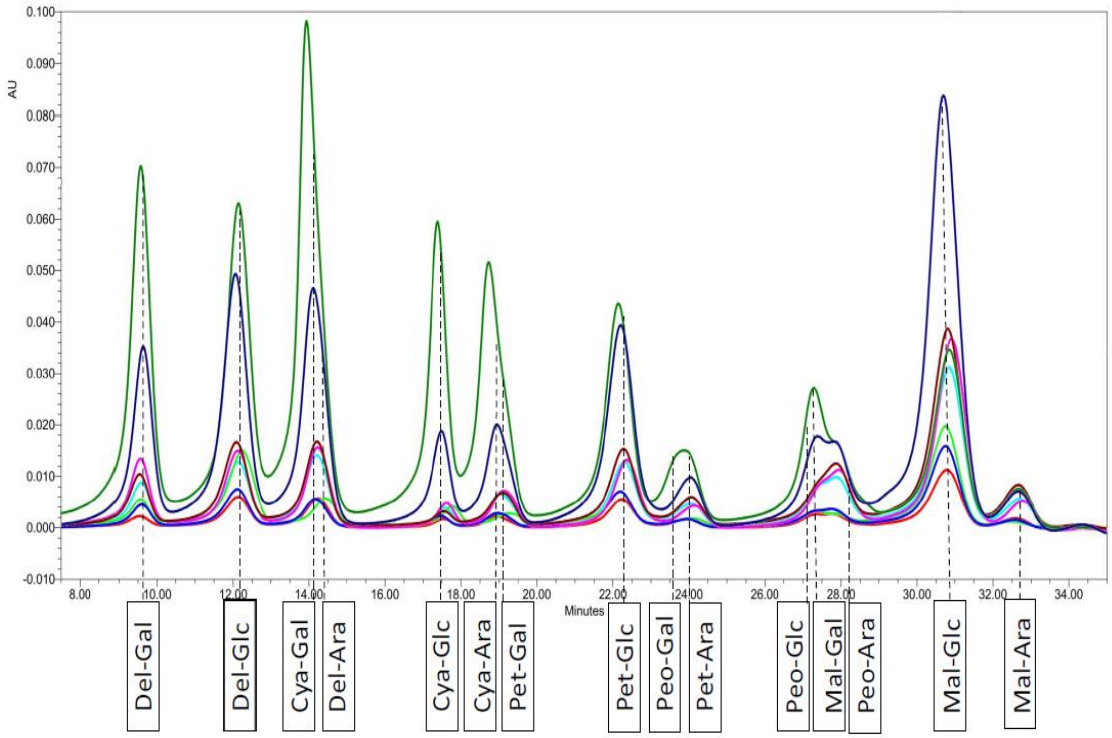

Figure 11: UPLC-DAD chromatogram overlay of all LLE extracts at 530 nm using System 2 with annotation

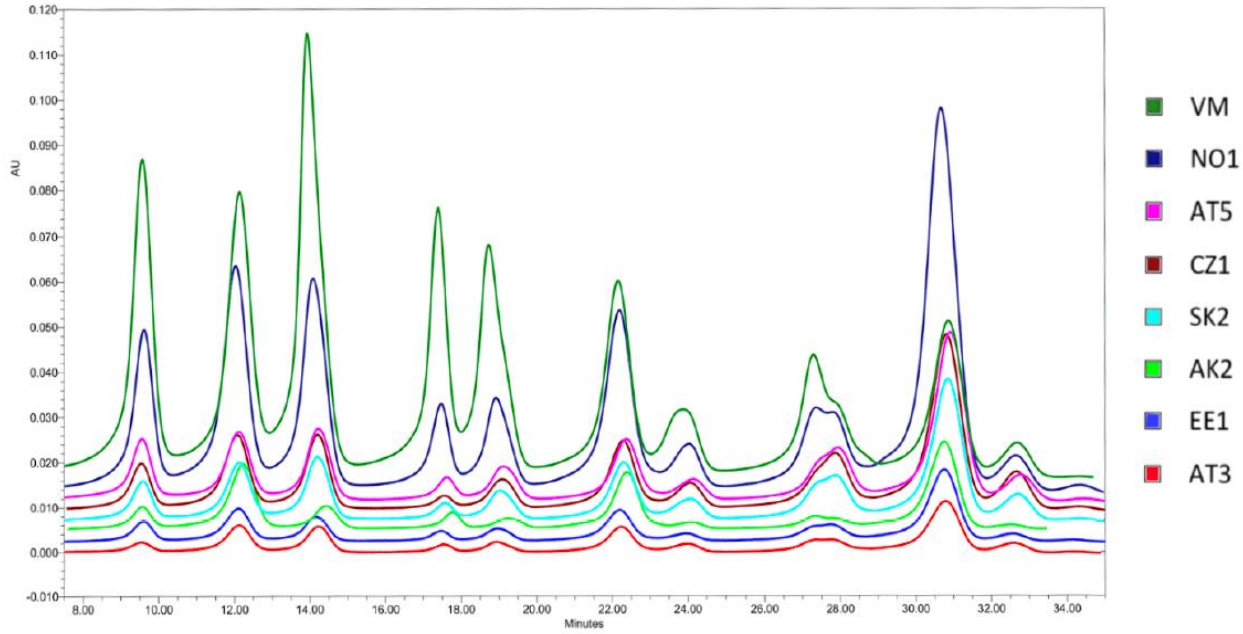

Figure 12: stacked UPLC-DAD chromatograms of all LLE extracts at 530 nm using System 2 with color legend

2.2 Effect of Fungal Infection on the Metabolite Content

To assess whether fungal infection influences the metabolic profile of *V. uliginosum* and therefore could be an explanation for the alleged toxicity of the fruits, a comparative analysis was made. The phytochemical comparison between healthy and *Monilinia megalospora*-infected berries of *V. uliginosum* showed clear differences (see Figure 14 and Figure 15). The synthesis of flavonoids appears to be reduced in the fungal infected berries, particularly the methylated quercetin derivatives such as myricetin and laricitrin. Anthocyanin production appeared to be almost completely inhibited in the infected berries. These findings are consistent with the spectrophotometric measurements of total soluble phenolics (TSP) and total anthocyanins (TMA) (see Figure 8).

Chemical dereplication of the LLE extract did not reveal any compound signals unique to the infected berry samples, suggesting that *Monilina* infection mainly influences the concentration of already existing metabolites rather than inducing new secondary metabolites.

Morphologically, *M. megalospora*-infected berries display an unappealing appearance, as shown in Figure 13. The fruits are pale, discoloured and display a whitish and shrivelled surface layer. Since anthocyanins are responsible for the characteristic red-purple-blue coloration of the peel, this visual discoloration supports the observed reduction in anthocyanin content described before (Kopystecka et al., 2023).

Figure 13: Comparison of healthy and *Monilinia megalospora*-infected berries of *V. uliginosum* from Alaska; healthy berries (left), infected berries (right)

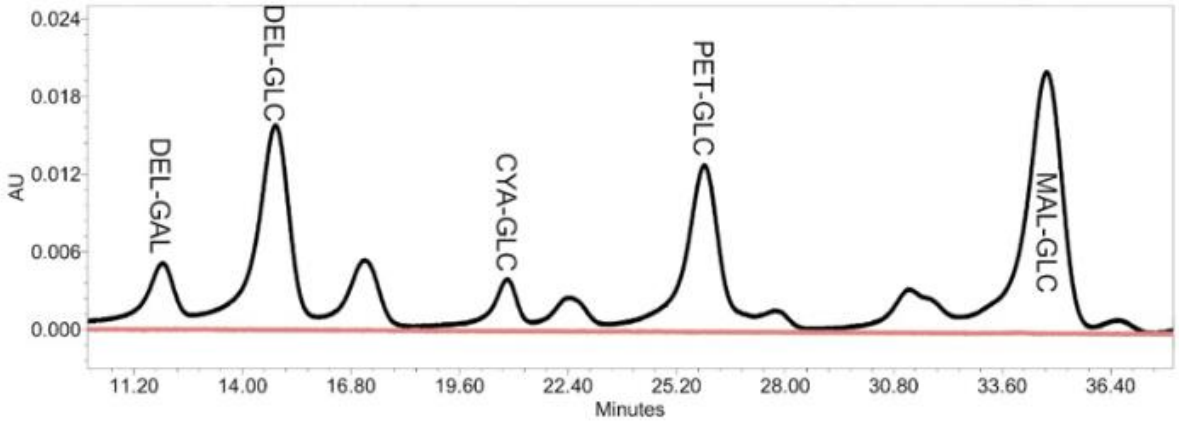

Figure 14: UPLC-DAD chromatograms of the LLE extracts from healthy (black) and infected (pink) berries at 530 nm using System 2

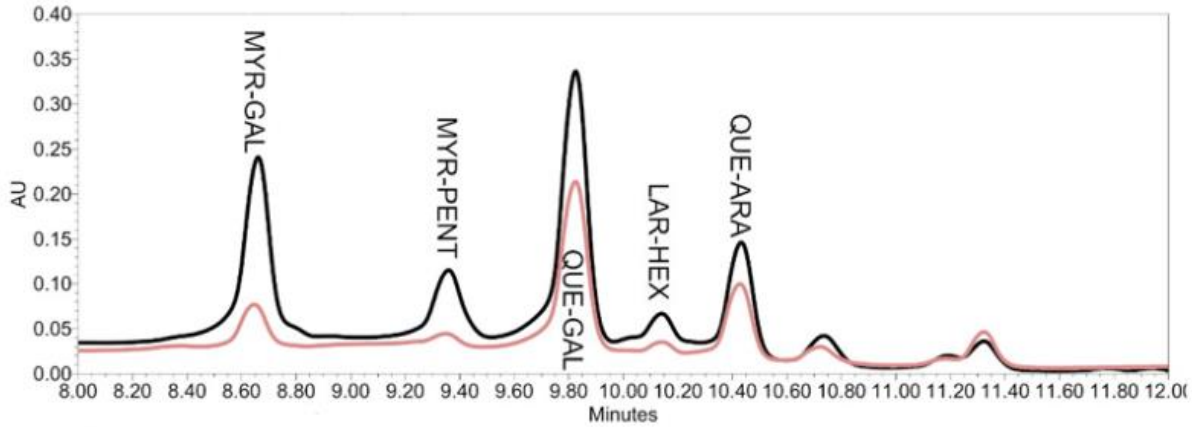

Figure 15: UPLC-DAD chromatograms of the LLE extracts from healthy (black) and infected (pink) berries at 370 nm using System 1

2.3 Bioactivity and Toxicity Evaluation

LLE extracts were chosen for bioactivity testing, since they are expected to best represent the chemical composition and potential biological impact that could occur after the ingestion of the berries (Camp et al., 2012). Extracts from the different berry samples were tested in two complementary bioassays assessing their effects on cell viability and survival rate. For the in vitro experiment, viability of the *Huh-7*-hepatocarcinoma cells was determined, while *C. elegans* was used as the in vivo model for the survival assays. Results are shown in Figure 16 and Figure 17. The depicted effects were achieved by applying relatively high concentrations of the extracts to the two assays, because the optimization process revealed that concentrations in nanogram/mL to low microgram/mL range achieved no effects in the chosen bioassay models (data not shown).

In the viability assay, treatment with 500 µg/mL of the extracts resulted in statistically significant, but only minor changes in cell viability compared to the control (Medium and Dimethylsulfoxide (DMSO)

0.5 %). No differences were observed between bog bilberry samples originating from different harvest locations, only the *V. myrtillus* extract produced a minor increase. The *Monilinia*-infected sample AK2M was tested repeatedly due to precipitation occurring at higher concentrations. Consequently, the apparent cytotoxic effect observed at the highest concentration may have been due to precipitation, as no significant decrease in cell viability was detected at lower concentrations where no precipitation occurred.

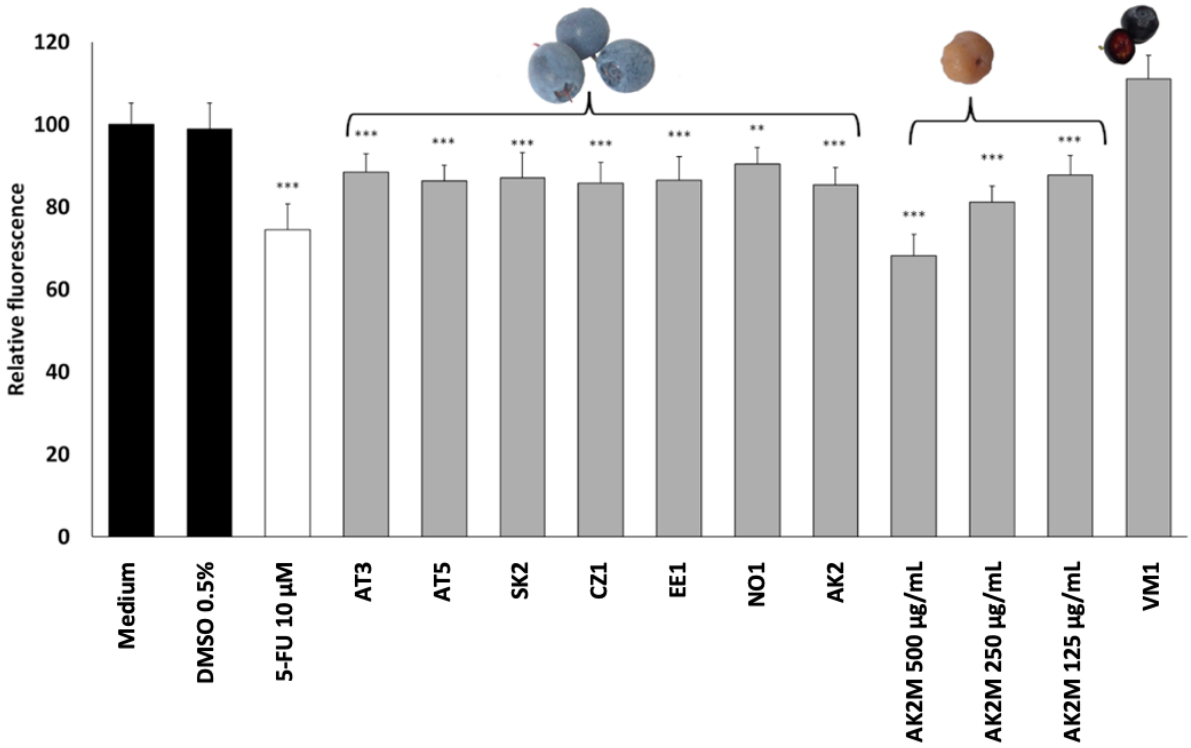

Figure 16: Cell viability of the Huh-7 hepatocarcinoma cells treated with LLE extracts of different berry samples at the concentration of 500 µg/mL, expressed as the relative fluorescence of a resazurin assay. Due to precipitation in the extract of the *Monilinia*-infected berries originating from Alaska was additionally tested at 250 and 125 µg/mL.

In the *C. elegans* survival assay, none of the extracts exhibited toxic effects, even at high concentrations of 300 µg/mL which were used in the experiment. Instead, a slight trend toward a prolonged nematode lifespan was observed, even though the effects were not statistically significant.

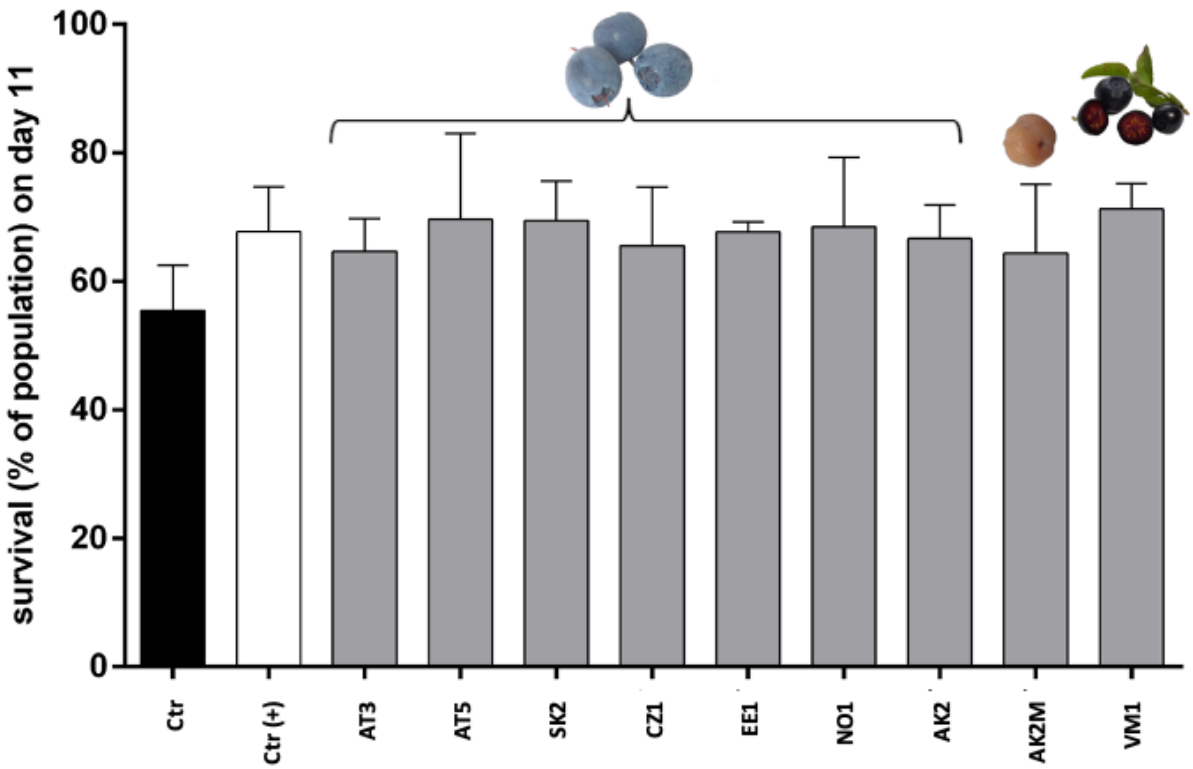

Figure 17: Survival of C. elegans populations on day 11 after treatment with LLE extracts of different berry samples at the concentration of 300 µg/mL

2.4 Fermentation of *V. uliginosum*

After the above-mentioned experiments ruled out direct toxicity in bog bilberries, we started looking into the fermentation capacity of *V. uliginosum* as an alternative explanation for the reported adverse effects. Bog bilberries have traditionally been used for preparing fermented beverages in several parts of the world (Vaneková et al., 2024). This practice might explain why the species is often referred to as "inebriating berry", "drunken berry" or "intoxicating berry" in several European languages (Netoliczky, 1914). To evaluate this hypothesis experimentally, a series of controlled fermentation experiments was conducted. The fermentation process was monitored by daily weight loss measurement, serving as an indirect measure of CO_2 release and fermentation activity (Liu et al., 2020).

In the first experiment, the fermentation potential of *V. uliginosum* was compared to two other *Vaccinium* species, *V. myrtillus* and *V. corymbosum*. The samples consisted of the raw berry purees without addition of sugar or yeast to imitate historic storage conditions mentioned in the earlier reports, which stated that bog bilberries ferments more efficiently than other berries.

As summarized in Table 2, the raw *V. corymbosum* juice generated the highest alcohol content by volume (ABV), which was to be expected due to its greater initial sugar content. In contrast, *V. myrtillus* proved to be unsuitable for spontaneous fermentation despite its initial relatively high °Brix value of 10, as the sample developed mold (see Figure 18) rather than undergoing alcoholic fermentation. *V. uliginosum* fermented moderately and without contamination, yielding a ABV percentage of 4.8 ± 0.4 %.

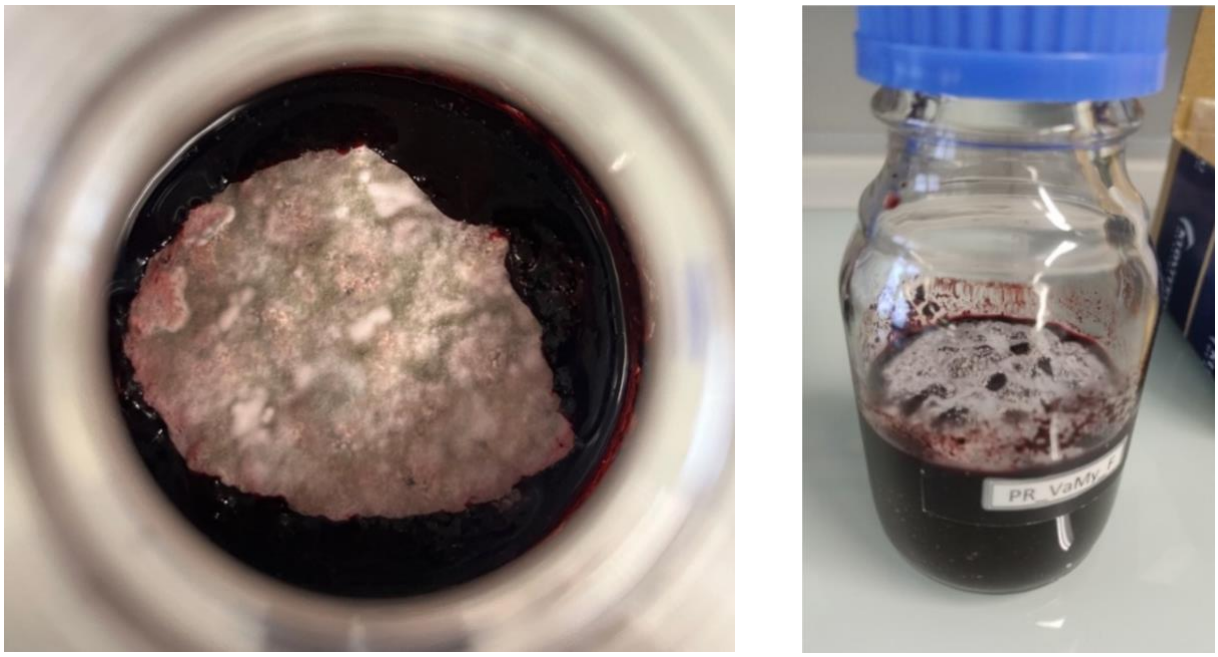

Figure 18: Mold in the fermentation bottle containing mashed *V. myrtillus* on day 5

Sensory differences between the fermented species were also notable. While *V. uliginosum* and *V. corymbosum* developed a pleasant, fruity aroma with a bright coloration, *V. myrtillus* samples looked duller in colour and exhibited an earthy, moldy smell.

Across all fermentation setups, pH values remained within the acidic range (2.83 – 2.97), which promotes yeast activity while inhibiting bacterial growth. Variations in pH affect which substrates microorganisms metabolize for energy, particularly the balance between malic acid and reducing sugars. These differences in the substrate for metabolization influence the degradation of organic acids and consequently the formation of volatile esters and alcohols that ultimately shape the aroma profile of the fermented berry wines (Wei et al., 2018).

Table 2: Physicochemical indexes of the different blueberry/bilberry species before and after fermentation

Species	Sample n = 1	pH	°Brix	ABV [%]
V. uliginosum	before	2.93	8	-
	after	2.91	5	4.8 ± 0.4
V. corymbosum	before	2.91	14	-
	after	2.97	4.8	13.2 ± 0.4
V. myrtillus	before	2.96	10	-
	after	2.83	9	1.75 ± 0.25

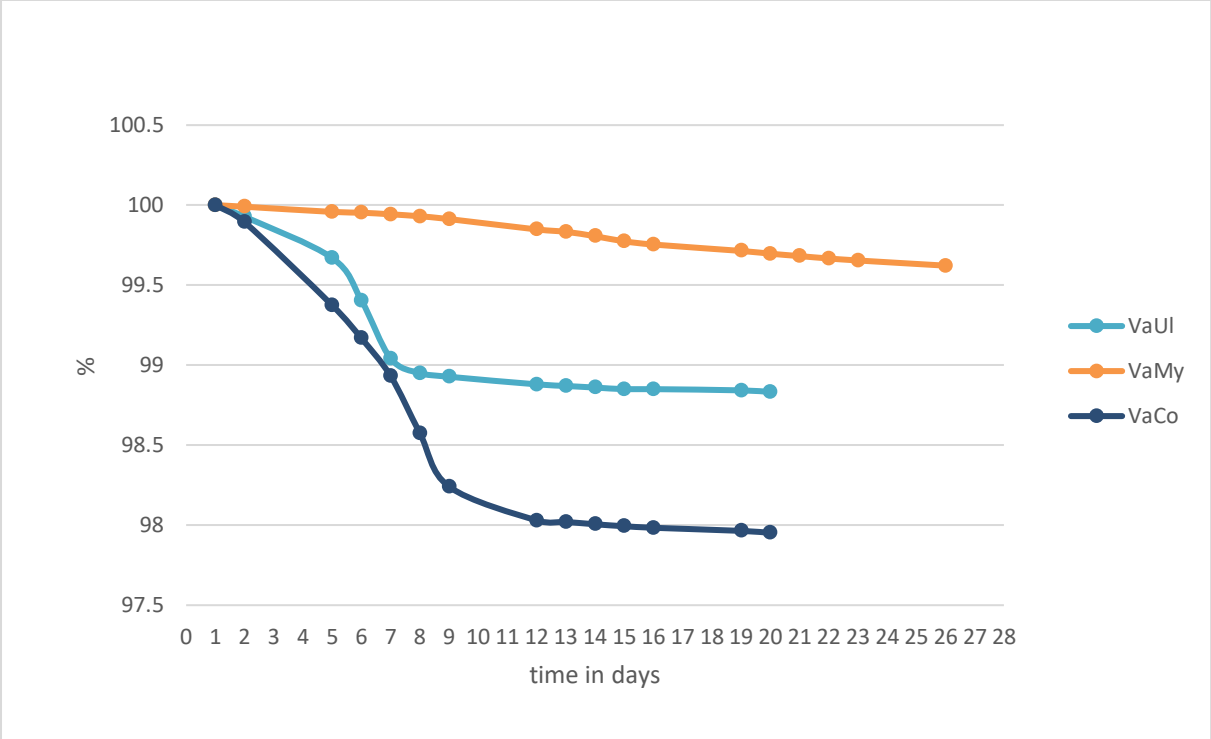

Figure 19: Weight loss monitoring of the fermentation process of different *Vaccinium* berry purees

In the second fermentation experiment, sugar addition and yeast inoculation were introduced into *V. uliginosum* juice to further refine the fermentation process (Table 3). The samples were labelled with "VUJ" for *V. uliginosum* juice, "S" for added sugar and "Y" for added yeast. Both treatment variations with added sugar (VUJS and VUJSY) produced higher ethanol yields than the other samples. As depicted in Figure 20, the addition of yeast accelerated the onset of fermentation. It also stands out that the VUJSY sample demonstrated a two-step fermentation kinetic; the reason for this is unclear.

Table 3: Physicochemical indexes of the *V. uliginosum* wines fermented under different conditions: VUJ: raw juice, VUJS: raw juice with added sugar, VUJY: raw juice with added yeast, VUJSY: raw juice with added sugar and yeast

Sample n = 2	Start °Brix	pH (before pH = 2.93)	°Brix after fermentation	ABV [%]
VUJ	8	2.84	5	5.2 ± 0.28
VUJS	16	2.86	9.2	8.2 ± 0.2
VUJY	8	2.81	4.8	7.3 ± 2.83
VUJSY	16	2.95	8.2	8.2 ± 1.82

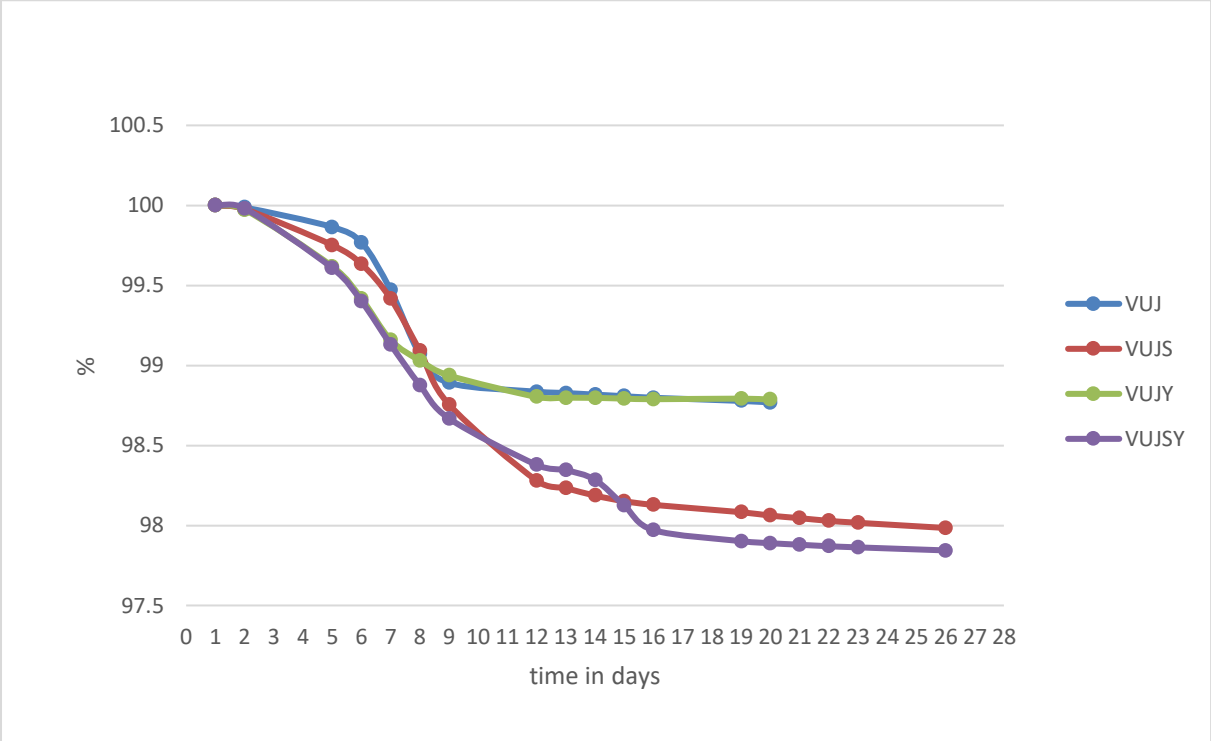

Figure 20: Weight loss monitoring of the fermentation process of *V. uliginosum* juice under different conditions

2.4.1 Alcohol Content

The ethanol content in the fermented berry samples was determined using two different approaches: (1) direct measurement through distillation and (2) indirect calculation based on the weight loss attributed to carbon dioxide release during fermentation. A comparative overview of both methods is provided in Table 4.

Table 4: Comparison of ABV values via distillation and via mass loss (*V. uliginosum*, *V. myrtillus* and *V. corymbosum* samples)

sample	**ABV via distillation** (average of samples) [%]	**ABV calculated via mass loss** (average of samples) [%]
Puree *V. uliginosum*	4.8 ± 0.4	4.0
Puree *V. myrtillus*	1.75 ± 0.25	1.3
Puree *V. corymbosum*	13.2 ± 0.4	7.1
Juice raw (VUJ)	5.8 ± 0.4	4.43 ± 0.06
Juice with sugar (VUJS)	8.2 ± 0.2	7.41 ± 0.57
Juice with yeast (VUJY)	7.3 ± 3.9	4.42 ± 0.26
Juice with sugar and yeast (VUJSY)	8.2 ± 2.2	8.07 ± 1.33

The most interesting finding was, that in all cases the distillation method yielded higher alcohol concentrations than the CO_2 based calculation. This might be explained by several factors. Firstly, calculations were made assuming an ideal conversion of 1 : 2 glucose to ethanol / CO_2 molar ratio during fermentation. Depending on the other constituents of the sample, the conversion can be more complex and therefore produce different outcomes. Moreover, underestimation of the ethanol production might be due to residual CO_2 in the sample containers. Some CO_2 might stay dissolved in the liquid or get trapped in foam or bubbles and not escape entirely by the end of fermentation.

Apart from the untouched puree, the samples with added sugar (VUJS, VUJSY) showed the highest ethanol concentrations as well as the best agreement between the two different methods. This suggests that extra sugar leads to a more complete fermentation. The sample "Puree *V. myrtillus"* yields the lowest ethanol content, by far, which was no surprise because the sample started to grow mold already on the second day (see Figure 18). The kind of mold genus is not exactly identifiable without microscopic analysis or further cultivation, however many mold types are known to compete with fermentative microbes and can therefore lower the sugar to alcohol conversion (Avîrvarei et al., 2023). The biggest discrepancy was seen in the *V. corymbosum* puree, where distillation showed 13.2 % but only 7.1 % via CO_2 loss, indicating either ethanol accumulation beyond CO_2 release or analytical loss in weight-based measurements.

2.4.2 Anthocyanins and Polyphenols During Fermentation

Figure 21 presents the TMA content of the first fermentation experiment, berry purees of the three different *Vaccinium* species, without any addition of sugar or yeast. Fermentation was stopped when they reached their weight loss maximum; VaCo and VaUl samples after 20 days, VaMy after 26 days. Clear differences in anthocyanin concentrations were observed. Before fermentation, the VaMy puree showed the highest anthocyanin content, reaching 1280.76 mg/mL berry juice equivalent. The anthocyanin concentration in VaUl was moderate at 628.49 mg/mL, whereas VaCo contained comparatively low levels with only 55.55 mg/mL.

The fermentation process decreased the anthocyanin content in two of the three samples. In VaMy, the concentration dropped to approximately half of the initial value, while VaUl also showed a notable reduction, although to a lesser extent. VaCo exhibited a minor increase, reaching now 74.44 mg/mL. This small increase may be explained by the initial extraction of pigments from the berry skins into the juice during early fermentation (Varo et al., 2022). However, because anthocyanins are relatively unstable compounds, they tend to degrade over time, resulting in lower TMA concentrations in later fermentation stages or as also observed in the other two samples (VaUl and VaMy).

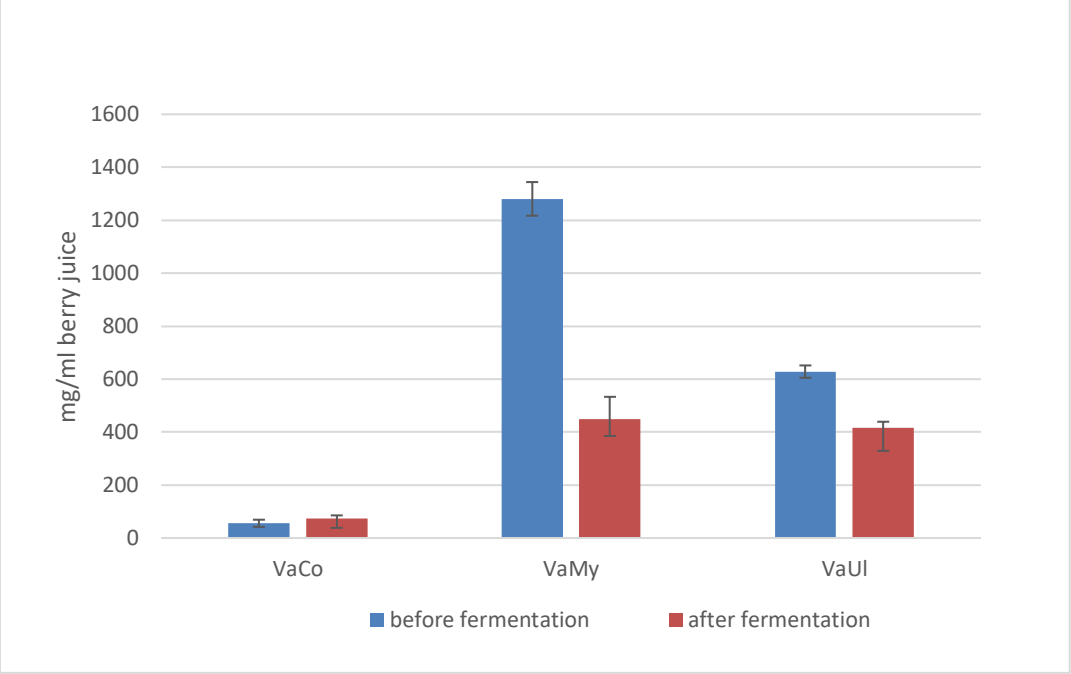

Figure 21: Anthocyanin quantification in different *Vaccinium* berry puree samples before and after fermentation

The total Polyphenol content, which also includes anthocyanins as one of its main subclasses, was also determined in the berry purees of VaMy, VaCo and VaUl before and after fermentation. Results are shown in Figure 22. Before fermentation, VaMy (3305.37 mg/mL) and VaUl (3054.07 mg/mL) contained far higher polyphenol levels than VaCo (269.78 mg/mL). After fermentation, an overall increase in total polyphenols was observed in all three species. This can be explained by enhanced extraction of phenolic compounds during fermentation, as cell disruption and ethanol formation promote their release from the berry tissue, or by the fact that the yeast cells themselves produce phenolic compounds as their metabolic byproducts during the fermentation process (Behrends & Weber, 2017). The biggest effect was exhibited in the *V. uliginosum* sample, where polyphenol content nearly doubled (5035.25 mg/mL). VaMy showed also showed a moderate increase, while VaCo displayed a comparably high relative increase with 809.08 mg/dl compared to its starting values.

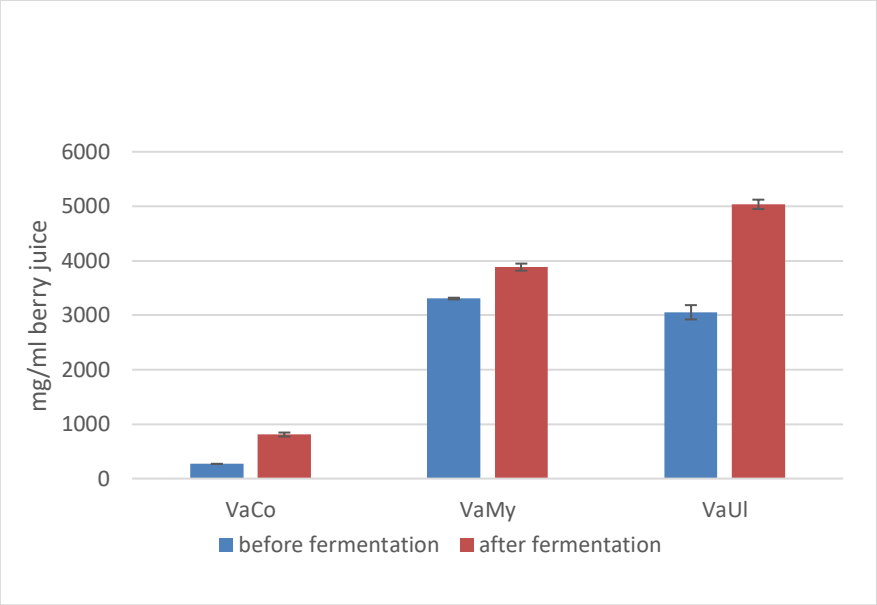

Figure 22: Polyphenol quantification in different *Vaccinium* berry puree samples before and after fermentation

The next illustration shows the comparison of TMA and TSP values of the samples of the second fermentation experiment (see Figure 23). Different batches of *V. uliginosum* juice were fermented under varying conditions, further described in chapter 4.5.2: without additives (VUJ), with added sugar (VUJS), with yeast (VUJY) and with both sugar and yeast (VUJSY). Each batch was prepared and analysed in duplicate and both replicates are shown separately to illustrate the variation within treatments. After fermentation, the resulting polyphenol concentrations ranged from 2563.28 mg/mL (VUJ_1) to 3862.09 mg/mL (VUJSY_2). The highest values were detected in a sample containing both yeast and added sugar, followed by a sample containing only added sugar. This is likely because the

added sugar provides the necessary fuel for the yeast to stay active longer, allowing for a greater production of phenolic metabolites. Lowest polyphenol values were noted in VUJ_1, the raw berry juice. In contrast, anthocyanin contents showed only minor variation between the treatments, reaching from 415.70 mg/mL (VUJ_2) to 476.20 mg/mL (VUJSY_1), with only one value escaping the range: 718.40 mg/mL (VUJSY_2).

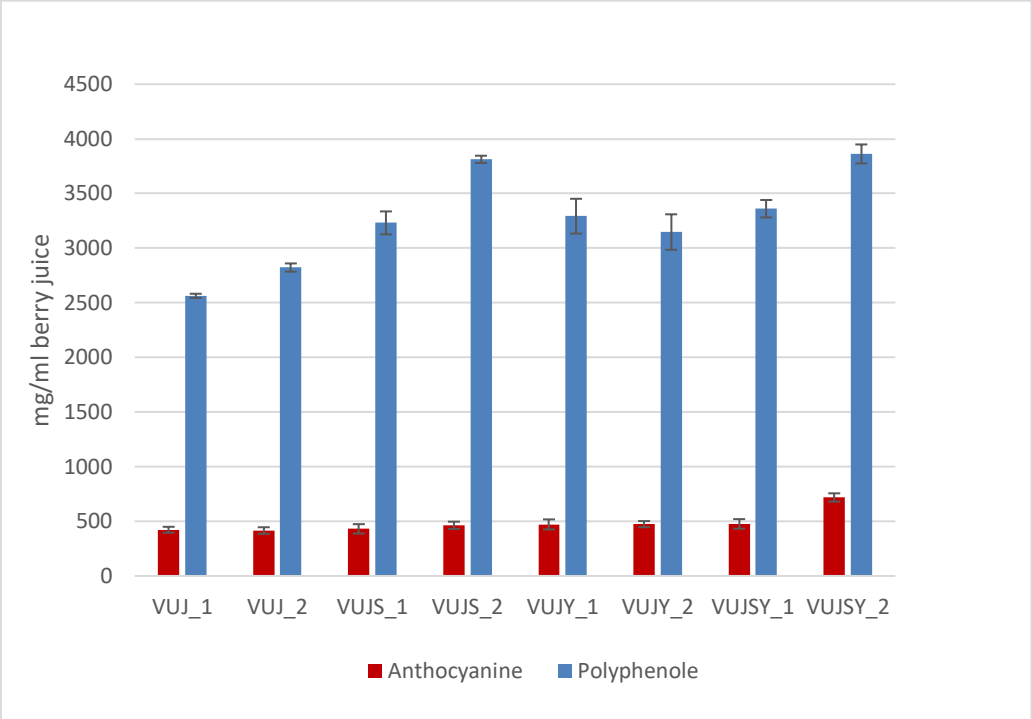

Figure 23: total anthocyanins and polyphenols in all *V. uliginosum* samples after fermentation

3 CONCLUSION

This master's thesis aimed to systematically investigate the phytochemical composition, biological activity and potential toxicity of *Vaccinium uliginosum* fruits to clarify their alleged toxicity and reported adverse effects following consumption. As part of a broader project called the "Bog bilberry Enigma", led by Zuzana Vaneková, some of the results obtained in this study were also included in a recent publication (Vaneková et al., 2025).

Across all analysed samples, the phytochemical composition was highly consistent. Although the absolute content of polyphenols and anthocyanins varied between samples from different geographical regions, no qualitative differences were noticeable between the European and North American populations. Importantly, no inherently toxic compound classes like alkaloids were detected. Likewise, berries collected from locations historically associated with reports of adverse effects did not show any distinctive chemical patterns. All in all, these findings suggest that the chemical variability of bog bilberry populations reflect environmental influences, such as climate and growing conditions, rather than the presence of location-specific toxic metabolites.

The biological assays executed in this study further support this conclusion. Extracts of bog bilberries did not exhibit relevant toxicity in either the in vitro Huh-7 liver cell model or the in vivo *Caenorhabditis elegans* assay. Only minor effects on cell viability were detected at very high extract concentrations, indicating a low toxic potential under the tested conditions.

Fungal infection by *Monilinia megalospora*, which has been one hypothetical explanation for a potential source of intoxication, did not result in the detection of any novel metabolites attributable to fungal biosynthesis. In the bioassays, the corresponding LLE extract did not show any significant differences compared to extracts from the healthy berry samples. Nevertheless, infected fruits displayed alterations in flavonoid and anthocyanin content. Given the low prevalence of infected fruits during sample collection and their unappealing appearance, it seems unlikely that fungal infections are the reason for historical poisoning events. As *Monilinia megalospora* infected berries were only available in a sizable amount from a single Alaskan location, the present findings are restricted. Future studies including infected material from European sites would be necessary to assess whether the observed effects are consistent across regions.

In contrast, the fermentation experiments provide the most plausible explanation for the widespread folklore. *V. uliginosum* fruits underwent spontaneous fermentation quickly and produced ethanol

concentrations of almost up to 5 % ABV, even without the addition of sugar or yeast. This aligns well with ethnobotanical reports of fermented berry products and suggests that accidental alcohol formation during storing or traditional processing could have significantly contributed to today's perception of the bog bilberry.

While this work aimed to specifically address the most frequently proposed hypotheses regarding the intoxicating effects of bog bilberries, it cannot exclude all possible factors. Individual sensitivity, rare intolerances or accidental co-consumption of other plant species may still account for the historical reports. Nonetheless, taken the present results it indicates that the bog bilberries themselves are unlikely to possess inherent toxic properties, but effects are much more likely to be caused by fermentation.

4 MATERIAL AND METHODS

4.1 Plant Material

Fully ripe *V. uliginosum berrie*s were collected by Zuzana Vaneková in different locations in Europe and North America between 2020 and 2023. Within 24 hours after picking, the fruits were frozen and stored until further preparation. 100 g of frozen berries from each batch were homogenized, freeze-dried and finely ground. The resulting berry powder was stored in a desiccator at room temperature until further analysis and labelled as stated in Table 5. Common bilberries (*V. myrtillus*) sourced from Slovakia were purchased from a local supplier and authenticated by Zuzana Vaneková, cultivated blueberries (presumably *V. corymbosum*) originating from Austria were obtained and processed identically to *V. myrtillus*. Berries of the deadly nightshade (*Atropa belladonna* L.) were harvested from the medicinal garden of the Faculty of Life Sciences, University of Vienna. They also were homogenized, freeze-dried, ground and then stored in a desiccator at room temperature until analysis, labelled as AtBe.

Further details regarding the collected samples are listed in Table 5 below. Sample names indicate the location and the sequential number of sampling. For the present analysis, a targeted selection of samples was made. This includes AT3 and EE1, which originate from locations associated with historical reports of bog bilberry toxicity (Nevinny, 1908; Zipf, 1944) as well as AK2M, a sample composed of *V. uliginosum* berries infected with *Monilina megalospora*. Other samples from the research project were not considered further because they were collected at less relevant sites or did not provide sufficient material for comprehensive analysis.

Table 5: List of *V. ulignosum* berry material and sample locations

Abbr.	Site characteristics					Collection date
	Country	GPS coordinates	Site name	Elevation [m a. s. l.]	Biotope	
AT3[1]	Austria	N 47.4591 E 12.3709	Schwarzsee, Kitzbühel	780	Peat bog by an alpine lake	08.09.2020
AT5	Austria	N 47.2933 E 11.7618	Kleiner Gammsstein	1900	Alpine blanket bog	11.08.2022
SK2	Slovakia	N 49.4294 E 19.4996	Klin bog	610	Peat bog	02.09.2020

CZ1	Czech Republic	N 48.8539 E 14.8352	Kočičí bláto	460	Dried peat bog	23.08.2022
NO1	Norway	N 69.6332 E 18.9950	Fløya, Tromsø	450	Tundra heath	11.09.2023
EE1[2]	Estonia	N 58.2564 E 27.0559	Võnnu	50	Clearing in pine forest	16.07.2023
AK2	Alaska, USA	N 69.9906 W 147.7928	Old Murphy Dome Road	530	Clearing in spruce forest	27.07.2023
AK2M[3]	Alaska, USA	N 69.9906 W 147.7928	Old Murphy Dome Road	530	Clearing in spruce forest	27.07.2023

[1] This site corresponds to the location reported in the bog bilberry toxicity case described by Nevinny (1908)

[2] This site represents the approximate location referenced in the bog bilberry toxicity case study by Zipf (1944)

[3] This sample consists of *V. uliginosum* berries infected with the fungus *Monilinia megalospora*.

4.2 Extraction Methods

4.2.1 Lead Like Enhanced (LLE) Extraction

The preparation of the LLE extracts followed a modified procedure based on the protocols of Camp et al. (2012) and Zwirchmayr et al. (2020). This technique was selected to obtain an extract enriched with as many orally bioavailable constituents as possible, while removing those that could give false positive results in bioassays (e.g. tannins) and minimizing all components which are not important for bioactivity testing (e.g. lipids and simple sugars) (Camp et al., 2012).

Approximately 600 mg of dried and finely ground berry material was weighed into a glass centrifuge tube. To defat the sample, 10 mL of n-hexane was added and the tube was sonicated for one minute, followed by 15 minutes of centrifugation at 3500 rpm. The supernatant n-hexane layers were collected separately in a pear-shaped flask. This defatting step was repeated for a second time. Next, 7 mL of dichloromethane CH_2Cl_2 was added to the fruit material for extraction, followed by 15 min of sonication in the ultrasonic bath. The mixture was centrifuged at 3500 rpm for 15 minutes. The supernatant was filtered through a cotton-plugged pipette and afterwards transferred into a round-bottom flask. To ensure complete extraction, the pipette was broken after the filtration and the cotton

wool was put back to the natural material. The extraction continued with the addition of 13 mL of MeOH. The sample was again sonicated in the ultra-sonic bath for 15 minutes and centrifuged for 15 minutes at 3500 rpm and 15 minutes of sonication in the ultrasonic bath. Lastly, the CH_2Cl_2 and MeOH extracts were combined and evaporated to dryness using a rotary evaporator.

For the removal of tannins, the dried extract residue was first redissolved in 4 mL of MeOH and then loaded onto a solid-phase extraction polyamide gel (CC-6; 900 mg, 20 µm frit) cartridge. The column was washed twice with 4 mL of MeOH and the combined MeOH eluates were concentrated under reduced pressure. The resulting extracts were transferred into pre-labeled, pre-weighed vials and left at the desiccator overnight before further analysis. See results in Appendix Table 14.

4.2.2 Alkaloid Extraction

For the purpose of performing TLC to investigate the alkaloid compounds of the sample, an extraction of each sample was carried out using a method adapted from the classical Stas-Otto procedure. About 2.00 g of the dried berry material was defatted with 10 mL n-hexane, placed in an ultrasonic bath for about 3 minutes and dried again under a soft stream of air under a fume hood. In order to liberate the alkaloid base, the plant material then got pre-treated with 1 mL of conc. ammonia. Before the next step, the samples were allowed to dry completely.

Subsequently, the sample material was extracted with 15 mL $CHCl_3$ in an ultrasonic bath for 15 min, then got placed on a shaker for another 15 min before getting filtered through a pipette stowed with cotton wool. The residue on the filter was washed with chloroform, then the procedure got repeated a second time. The combined $CHCl_3$ filtrates were collected in a round flask and were evaporated to complete dryness on a rotary evaporator.

First, the residue in the round flask was dissolved in 15 mL 1N H_2SO_4 and then was neutralized with conc. ammonia to pH 9 - 10. Afterwards, the extract was transferred to a separatory funnel and shaken twice with each 10 mL of chloroform. The two resulting $CHCl_3$ layers were combined and reduced to a lower volume on the rotary evaporator before finally getting transferred to a pre-weighed and labelled vial and left to dry completely under the sample concentrator.

In preparation for the TLC, the extracts were dissolved in 0.5 – 1.5 mL MeOH depending on the amount of extract (see table in Appendix Table 13).

4.3 Analytical Methods

4.3.1 Chromatographic Methods

Chromatography is an analytical technique that separates compounds based on their differential interactions between a stationary and a mobile phase. These interactions depend on the physicochemical properties of each analyte and result in characteristic migration or retention behaviours. Depending on the chromatographic system, compounds can be distinguished by their retention time, peak pattern or migration distance, which helps with qualitative identification (Gey, 2008). In this work, three chromatographic techniques (TLC, UPLC and SFC) were used, each of which will be described in the following chapters.

4.3.2 TLC Systems

For the analysis of the alkaloid extracts, TLC was carried out. The extracts were reconstituted in MeOH before applying them onto the stationary phase, which consisted of Silica gel 60 F_{254} plates. To ensure optimal conditions for analyte separation and detection, four plates containing identical sample sets were prepared and developed separately under different conditions. Two different kinds of mobile phases were used, with two plates each being developed under chamber saturation (see Table 6). Afterwards, two different sets of spraying reagents were applied: one plate of each mobile phase was treated with Dragendorff reagent, while the other was derivatized using vanillin and sulfuric acid.

For the first detection method, Dragendorff reagent was applied to the plate by spraying and prepared as follows: 5 mL of Solution I was mixed with 5 mL of Solution II, before adding 20 mL of conc. acetic acid and 100 mL of H_2O (Solution I: 0.85 g basic bismuth nitrate in 10 mL conc. acetic acid + 40 mL of H_2O, Solution II: 8.0 g potassium iodide in 20 mL H_2O). As for the second detection method, the plates were sprayed with a 1 % solution of vanillin in MeOH and afterwards a solution with 5 % H_2SO_4 in MeOH, followed by heating to approximately 105 °C for 1 - 3 minutes. Visualization of the resulting spots was carried out under both visible and ultraviolet light.

Table 6: Parameters used for TLC screening

Mobile Phase	70:20:10 (Toluene – Ethyl acetate – Diethylamine)
	1:1 (Chloroform – Acetone)
Stationary Phase	TLC Silica gel 60 F254
Spraying reagents	Dragendorff reagent
	Vanillin in MeOH and H_2SO_4 in MeOH
Detection	UV254, UV366, visible light

4.3.3 UPLC-DAD-MS

To analyze the secondary metabolites of the LLE extracts, a Waters Acquity UPLC instrument consisting of a sample-, quaternary solvent- (QSM), isocratic solvent- (ISM) and column manager as well as a photodiode array (PDA) and Quadrupole Dalton (QDa) detector was used. For QDa operation nitrogen served as nebulizing gas. The operating software used was Empower 3. The UPLC methods and specific parameters used are shown in Table 7, Table 8, Figure 24 and Figure 25.

Table 7: UPLC-PDA-QDa method System 1

Instrument	ACQUITY UPLC H-Class System by Waters			
Column	ACQUITY BEH C18 (2.1 x 100 mm, 1.7 µm)			
Flow rate [mL/min]	0.3			
Temperature	45°			
Detectors	PDA, QDa			
PDA [nm]	detection wavelength 370 nm for flavonoids, 530 nm for anthocyanins			
Isocratic Solvent Manager (ISM)	Flow rate [mL/min]	Solvent		
	0.4	90 % H2O + 10 % MeOH with 10 mM ammonium formate		
QDa detector settings	capillary voltage 0.8 kV (positive and negative), cone voltage 15 V (positive) and 30 V (negative), mass range 100 – 1000 m/z, probe temperature 600 °C			
Mobile phase A	Water + 0.1 % formic acid			
Mobile phase B	Acetonitrile + 0.1 % formic acid			
Mobile Phase C	Methanol + 0.1 % formic acid			
Gradient	Time [min]	A [%]	B [%]	C [%]
	0.0	98.0	0.0	2.0
	1.0	98.0	0.0	2.0
	2.0	85.0	0.0	15.0
	15.0	40.0	0.0	60.0
	16.0	5.0	0.0	95.0
	18.0	2.0	98.0	0.0
	22.0	2.0	98.0	0.0
	22.5	98.0	0.0	2.0
	24.0	98.0	0.0	2.0

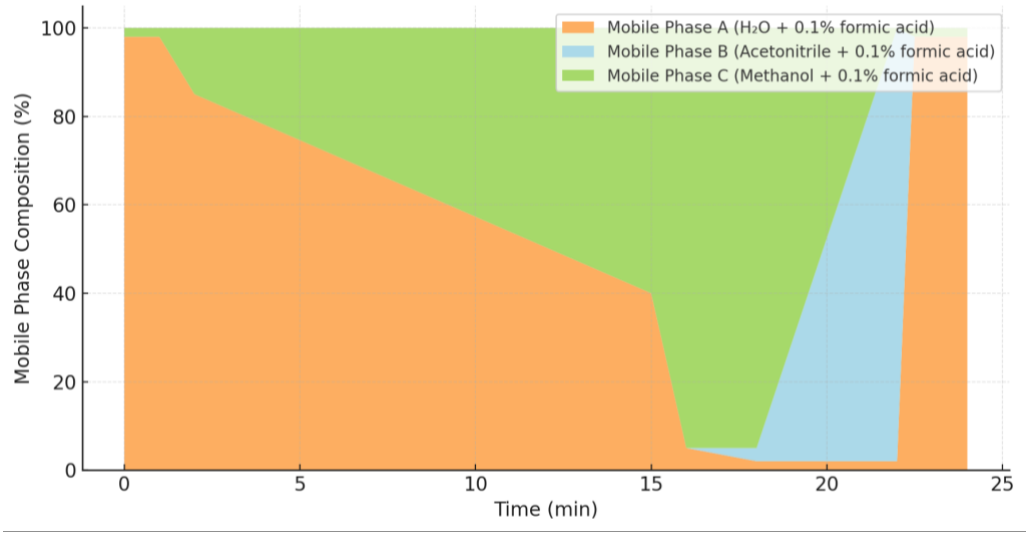

Figure 24: Gradient UPLC method 1

Table 8: UPLC-PDA-QDa method System 2

Instrument	ACQUITY UPLC H-Class System by Waters		
Column	ACQUITY BEH C18 (2.1 x 100 mm, 1.7 µm)		
Flow rate [mL/min]	0.3		
Temperature	50°		
Detectors	PDA, QDa		
PDA [nm]	detection wavelength 370 nm for flavonoids, 530 nm for anthocyanins		
Isocratic Solvent Manager (ISM)	Flow rate [mL/min]	Solvent	
	0.4	90 % H2O + 10% MeOH with 10 mM ammonium formate	
QDa detector settings	capillary voltage 0.8 kV (positive and negative), cone voltage 15 V (positive) and 30 V (negative), mass range 100 – 1000 m/z, probe temperature 600°C		
Mobile phase A	Water + 1 % formic acid		
Mobile phase B	Methanol + 1 % formic acid		
Gradient	Time [min]	A [%]	B [%]
	0.0	93.0	7.0
	43.0	78.0	22.0
	43.5	5.0	95.0
	48.0	5.0	95.0
	48.5	93.0	7.0
	50.0	93.0	7.0

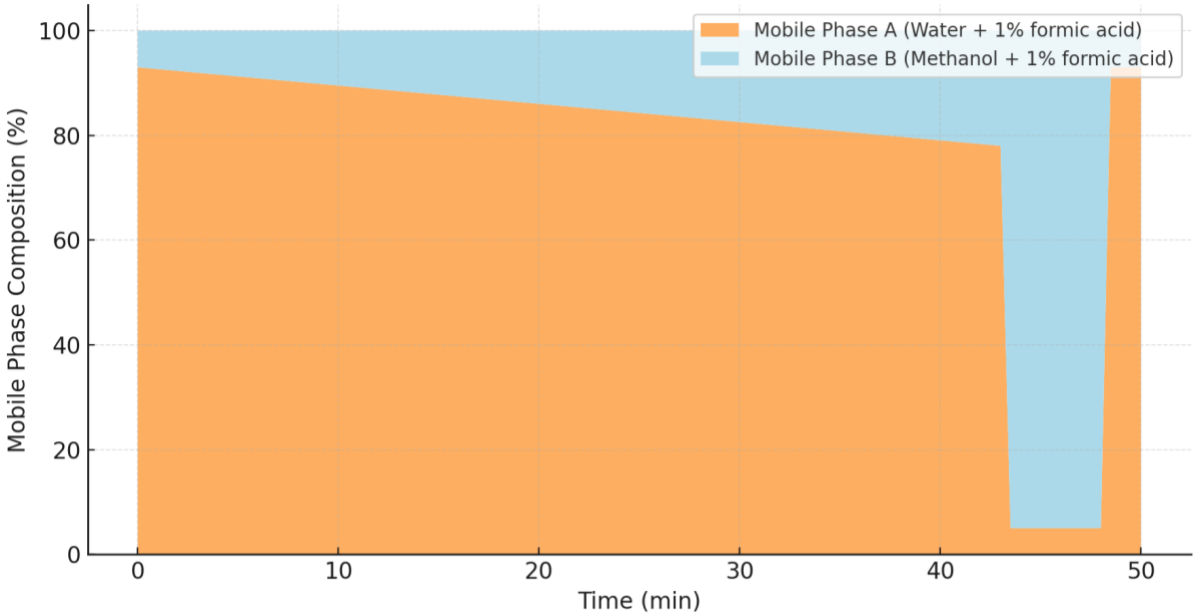

Figure 25: Gradient UPLC method 2

4.3.4 UHPSFC-DAD-ELSD-MS

To investigate the nonpolar constituents of the Stas-Otto extracts, a Waters Acquity UPC^2 system was used. The instrument included a convergence module, sample manager, binary and isocratic solvent managers and a column manager. The system was equipped with photodiode array (PDA), evaporative light scattering (ELSD) and Quadrupole Dalton (QDa) detectors. Nitrogen functioned as the nebulizing gas for both QDa and ELSD. Empower 3 was used for system as operating software. The setting for the analytical instruments are depicted in Table 9 and Figure 26.

Table 9: Settings UHPSFC-DAD-ELSD-MS for analyzation of nonpolar constituents

Instrument	ACQUITY UPC_2 System by Waters		
Column	Torus 1-AA (1-Aminoanthracene), 130 Å, 1.7 µm, 3 x 100 mm		
Injection volume [µl]	4 µL		
Flow rate [mL/min]	1 mL / min for both BSM and ISM Backpressure: 2000 psi		
Temperature [°C]	50°C		
Detectors	PDA, ELSD, QDa		
PDA [nm]	detection wavelength 210 nm		
ELSD	detector gain 100, gas pressure 40.0 psi, nebulizer in heating mode drift tube temperature 50.0 °C		
QDa	capillary voltage 0.8 kV (positive and negative), cone voltage 15 V (positive) and 30 V (negative), mass range 100 – 1000 m/z, probe temperature 600 °C		
Mobile Phase A	supercritical CO_2		
Mobile Phase B	methanol		
Gradient	Time [min]	A [%]	B [%]
	0.0	95.0	5.0
	3.0	90.0	10.0
	10.5	74.0	26.0
	11.0	95.0	5.0
	12.0	95.0	5.0

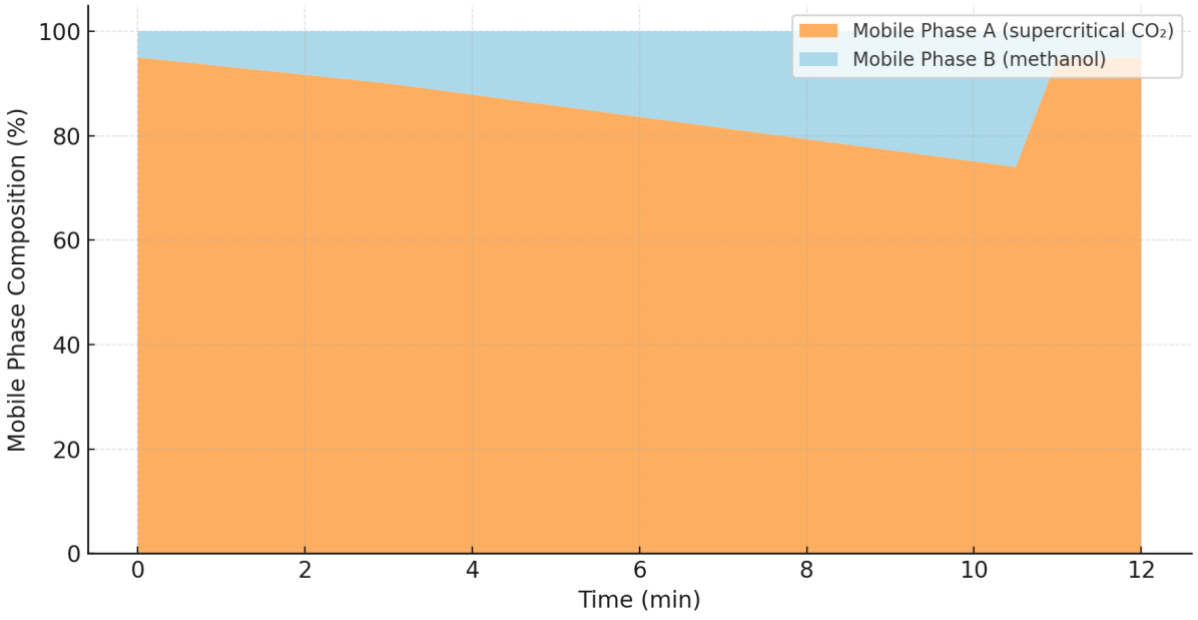

Figure 26: Mobile phase gradient of supercritical CO_2 (A) and methanol (B) for UPC^2 screening

4.3.5 Spectrophotometric Assays

Total soluble polyphenols

Total soluble polyphenols were spectrophotometrically determined following a protocol from Doumett (2011), using (+)-catechin as a reference standard. As a first step, methanolic sample solutions with a concentration of 10 mg/mL were prepared from the previously obtained LLE extracts. 100 µl of these extract solutions were mixed with 200 µl of Folin-Ciocalteu's (F-C) phenol reagent in a volumetric flask. After three minutes, 400 µl of an aqueous solution saturated with sodium carbonate were added. The obtained mixture was filled up to the 10 mL mark with doubled distilled water (ddH2O). The solution was incubated in the dark for one hour and afterwards the absorbance was measured at 740 nm. For each sample, a compensation solution was prepared following the same protocol, the only difference being that no F-C reagent was added. When measuring with the UV-spectrometer the compensation solution was measured first, followed by the sample solution. Each sample was prepared in triplicate and then measured three times each. The polyphenol concentration was calculated based on a catechin calibration curve and results were expressed as milligrams of (+)-catechin per 100 g of dry weight berries.

For the calibration curve, 6 methanolic (+)-catechin solutions ranging from 0.0625 mg/mL to 1 mg/mL were measured. Results can be seen in Figure 27.

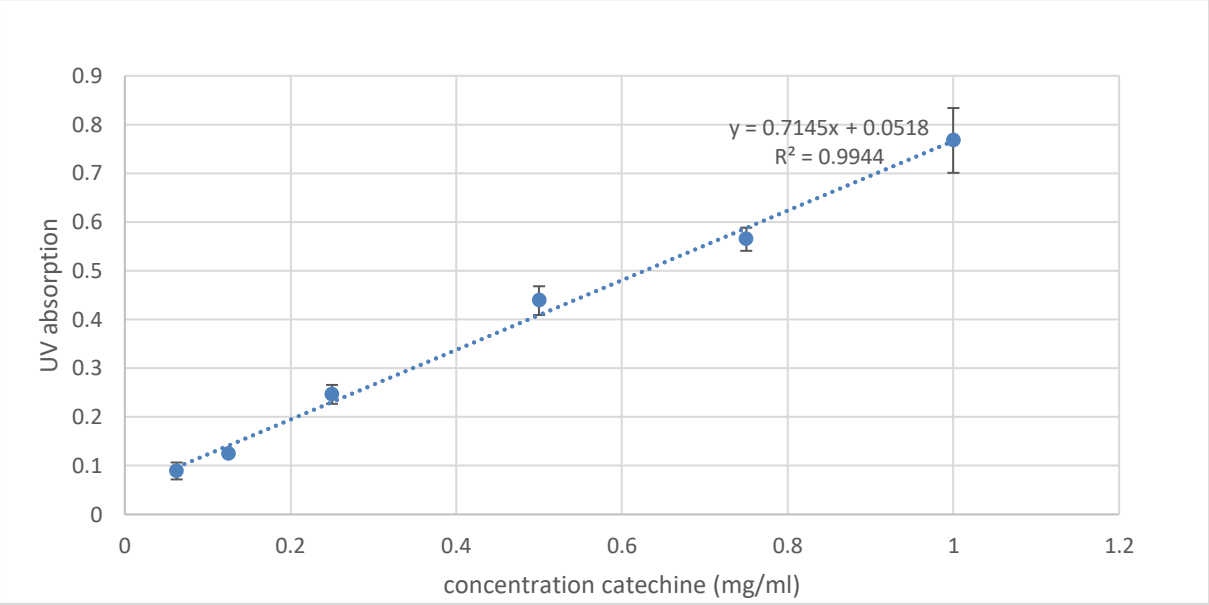

Figure 27: Calibration curve catechin used for polyphenol quantification (R2 = 0.9944)

Total monomeric anthocyanins

The UV-spectrometer was also used to determine the amount of anthocyanins, in accordance to a protocol from Lee et. al. (2005). Methanolic sample solutions with a concentration of 10 mg/mL were prepared from the previously obtained LLE extracts. For each sample, aliquots of 100 µl of berry extract solution were diluted in buffer solutions at pH = 1 and pH = 4.5 in a volumetric flask to obtain a final volume of 10 mL. The diluted test portions were read versus a blank cell filled with the buffer solution. The absorbance (Abs) of each solution was measured at 520 nm and within 20 – 50 min of preparation. The quantity was calculated using the following equation.

$$\Delta Abs = (Abs_{pH=1}^{520nm} - Abs_{pH=1}^{700nm}) - (Abs_{pH=4.5}^{520nm} - Abs_{pH=4.5}^{700nm})$$

As a last step, results were determined using a CYA-3-GLU calibration curve displayed in Figure 28.

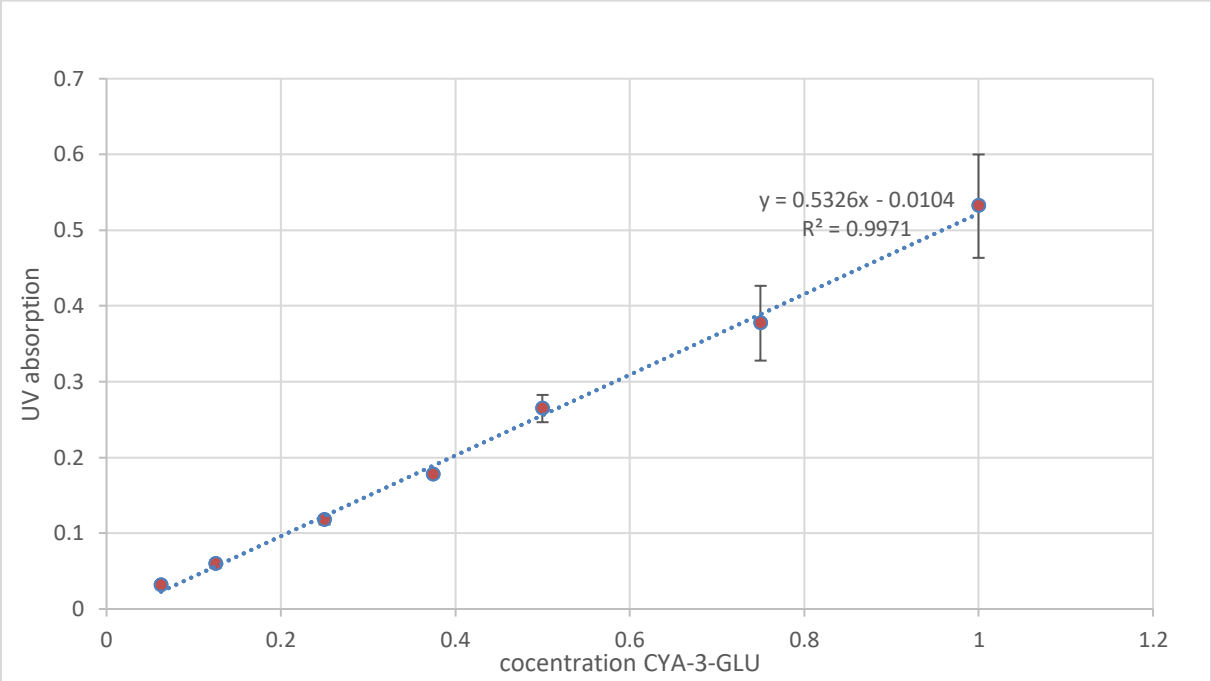

Figure 28: Calibration curve CYA-3-GLU used for anthocyanin quantification (R^2 = 0.9971)

4.4 Bioassays

To assess the potential biological effects of the berry extracts one in vivo and one in vitro bioassay were applied to the LLE extracts.

Cell viability assay

Cell viability was determined using the resazurin reduction assay and Huh-7 hepatocarcinoma cells. In viable cells, resazurin is reduced by the intracellular redox reactions involving cofactors such as NADH, forming the fluorescent metabolite resuforin. Fluorescence intensity, which is the reflection of metabolic activity, was measured at an excitation wavelength of 535 nm and an emission wavelength of 590 nm using an TECAN Spark Cyto plate reader.

Huh-7 cells were obtained as a gift from P. Blattmann (Institute of Molecular Systems Biology, ETH Zurich, Switzerland) and cultured in RPMI-1640 medium (Sigma-Aldrich, cat. No. R8758) supplemented with 5 % dialyzed fetal bovine serum (Fisher Scientific, cat. no. 10594553) and 1 % penicillin-streptomycin (Sigma-Aldrich, cat. no. P4333). About 24 hours before the treatment with the LLE extracts, cells were seeded in 96-well plates (Fisher Scientific, cat. no. 10212811) at a density of 2 x 10^4 cells/mL, dispensing 150 µL per well. The plates were incubated overnight at 37 °C with 5 % CO_2 to allow cell attachment and equilibration.

Bog bilberry extracts were tested in triplicate at a final concentration of 500 µg/mL. For the *M. monilina*-fungus infected berry sample from Alaska (AK2M), additional concentrations of 250 µg/mL and 125 µg/mL were included, because precipitation was occurring at higher concentrations. Cells treated with DMSO 0.5 % (Sigma-Aldrich, cat. no. D2650) served as vehicle controls, while 10 µM 5-fluorouracil (5-FU) (Sigma-Aldrich, cat. no. F6627) was used as a positive control for toxicity.

At 96 hours of incubation with the different extracts, resazurin (Sigma-Aldrich, cat. no R7017) was added to each well with a final concentration of 10 µg/mL, followed by 2 hours of incubation with 37 °C in 5 % CO_2 atmosphere. Fluorescence was then measured and cell viability was expressed as a percentage relative to the untreated control. Statistical evaluation was performed using the Student's *t*-test, with significance defined at $p \leq 0.05$.

***Caenorhabditis elegans* survival assay**

Wild-type *Caenorhabditis elegans* (strain N2, var. Bristol) and *Escherichia coli* OP50 were obtained from the *Caenorhabditis* Genetics Center (University of Minnesota, USA). Nematode growth medium (NGM) plates and s-complete medium were prepared according to standard procedures (Stiernagle, 2006) and the maintenance of both *C. elegans* and *E. coli* OP50 cultures followed the protocol described by Zwirchmayr et al (2020). *E. coli* OP50 was grown in LB medium at 37 °C for 8 hours, harvested by centrifugation and washed twice with double distilled water. The bacterial pellet was air-dried, then resuspended in S-complete medium at a concentration of 100 mg/mL and stored at 4 °C until required. *C. elegans* cultures were maintained on NGM agar plates seeded with OP50 at 16 °C. Transfers to freshly seeded plates were performed weekly to maintain healthy populations, while monitoring routinely. Three days prior to synchronization, worms were transferred to fresh NGM plates for the survival assay.

96-well plates were stored at a temperature of 25 °C during the experiment. The *C. elegans* survival assay was performed according to a method described in a publication from Redl et al. (2024). For synchronization, adult worms were collected and suspended in double-distilled water, afterwards exposed to an alkaline hypochlorite (bleaching) solution for 5 - 10 minutes to dissolve the adult cuticle and release eggs. The reaction was stopped with M9 buffer. Collection of the eggs was achieved by centrifugation at 2500 x g. Washing was done twice with M9 buffer and once with S-complete medium. Eggs were incubated in S-complete at room temperature for 48 hours to allow hatching and development to the L1 larval stage.

Between 5 and 20 synchronized L1 larvae were distributed into the inner wells of three 96- well plates. The outer wells were filled with S-complete medium to prevent diffusion. Worms were fed with *E. Coli* OP50 (6 mg/mL) and incubated at 25 °C. After 24 hours, reproduction was suppressed by adding 5-fluorodeoxyuridine (final concentration 0.12 mM; Sigma-Aldrich). Young adult worms were then exposed to the LLE samples, 100 µM cyanidine chloride (positive control) or 0.7 % DMSO (vehicle control). Each condition was tested in triplicate and repeated in three independent experiments. On the fourth day of adult lifespan, worms were supplemented with 3.8 % µL of OP50 suspension (100 mg/mL) to avoid starvation. Survival was assessed on day 11 under a dissecting microscope. Worms were classified as dead if they failed to respond to mechanical touch or light stimuli. Survival rates were calculated as the percentage of living worms relative to the total number of worms per condition, expressed as mean ± standard derivation (SD). Data visualization was performed using GraphPad Prism 9 and statistical analysis was conducted by one-way ANOVA followed by Dunnett's post hoc test, considering differences significant at $p \leq 0.05$.

4.5 Fermentation Experiments

4.5.1 Preparation of Fermentation Batches

Frozen berry samples of *Vaccinium uliginosum*, *V. corymbosum*, and *V. myrtillus* were thawed at room temperature for approximately 4 – 5 hours prior to further processing. Once thawed, the samples were homogenized using a hand blender. For each *Vaccinium* species, an initial portion of the puree was directly transferred into a fermentation vessel. For *V. uliginosum*, the remaining puree was centrifuged at 3500 rpm for 10 minutes to obtain the fruit juice, which was then used for the following fermentation experiment.

As shown in Figure 29, four different fermentation conditions were established for *V. uliginosum* juice: (1) without the addition of sugar or yeast, (2) with yeast only, (3) with sugar only, and (4) with both sugar and yeast. Each of these four conditions was carried out in duplicate, resulting in a total of eight fermentation vessels for the berry juice. Including the three puree-based fermentation vessels (one per species), a total of 11 fermentation vessels were monitored simultaneously throughout the fermentation process.

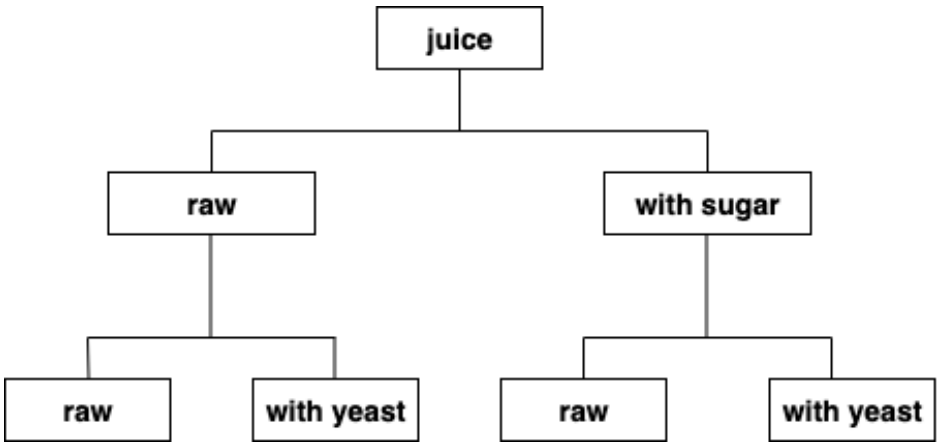

Figure 29: Fermentation conditions overview

4.5.2 Addition of Sugar and Yeast

Oenoferm X-thiol yeast (*Saccharomyces cerevisiae* Oenoferm® X-thiol, Erbslöh Geisenheim AG, Geisenheim, Germany) is a hybrid yeast strand derived from the crossing of two *Saccharomyces cerevisiae* strains and was selected for this experiment due to its favorable characteristics, like a tolerance to lower temperatures. With an optimum of 15 – 22 °C according to the manufacturer's

website, fermentation vessels can be left at normal room temperature throughout the process without additional temperature control (erbsloeh.com, last visited 12.11.2025).

The yeast starter culture was prepared as follows: 0.5 g of yeast was rehydrated with about 5 mL of 30 °C water and left for a few hours at room temperature until visible activation occurred, indicated by bubble formation. Afterwards, the yeast water mixture was combined with the equal amount of berry juice (5 mL with a brix value of 16°). From this suspension, 2.5 mL was added to the intended fermentation vessels according to the scheme.

For the fermentation containers requiring added sugar, the amount was calculated to adjust the berry juice to a final Brix value of 16 °, ensuring a standardized initial sugar concentration.

4.5.3 Fermentation Setup and Monitoring Design

The fermentation setup consisted of sterilized 250 mL glass laboratory bottles fitted with airtight blue screw caps. Each cap was modified to accommodate a fermentation lock, commonly used in small-scale fermentations. The airlocks were S-shaped plastic water locks, filled partially with sterile water and sealed at the top with caps to prevent contamination while allowing gas to escape (pictured below Figure 30).

Figure 30: Fermentation set up

This design allows carbon dioxide (CO_2) generated during fermentation to escape while simultaneously preventing the entrance of oxygen or airborne contaminants, thereby maintaining anaerobic conditions. The buildup and release of CO_2 can be visually monitored through bubbling activity within the airlock, serving as an indicator of ongoing fermentation.

The experimental design was based on the principle that yeast (*Saccharomyces cerevisiae*) metabolizes sugars into ethanol and carbon dioxide. As CO_2 gas escapes through the airlock during fermentation, the total mass of the vessel decreases over time. This mass loss was used as an indirect measure of fermentation progress. To monitor this, each vessel was weighed daily under consistent conditions. All bottles were kept at room temperature (approx. 22 °C). Fermentation was considered complete and the experiment stopped once the weight remained constant (i.e, < 0.1 g weight loss) at two consecutive monitoring time points, indicating that CO_2 release and therefore sugar conversion had reached its limit.

4.5.4 Analytical Methods Used in the Fermentation Experiment

The following analytical methods were applied to assess the physicochemical changes occurring during the fermentation experiment.

pH-Measurement

The pH of all berry juice samples was each measured twice, directly before and after fermentation. A standard glass pH meter was used and calibrated prior to measurement with buffer solutions of pH 4.00 and 7.00 according to the manufacturer's guidelines (HI223, Hanna Instruments, 650 Germany). To prevent cross-contamination, the electrode was rinsed with distilled water and gently blotted dry between measurements.

UV-Vis analysis of polyphenols and anthocyanins

Upon completion of the fermentation process, all fermentation batches were centrifuged, and the resulting supernatants were collected for subsequent analyses. The determination of total polyphenols and monomeric anthocyanins was carried out following the protocols described in Chapter 4.3.5, "Total Soluble Polyphenols" and "Monomeric Anthocyanins," using a UV-Vis spectrophotometer (Shimadzu UV-1800, Serial No. A116353).

For *V. uliginosum* samples, a volume of 50 µL of juice was used for anthocyanin quantification and 25 µL for polyphenol analysis. Higher volumes (e.g., 100 µL) led to absorbance values exceeding the limits of the calibration curve. Quantitation was performed using the same calibration curves applied to the unfermented *V. uliginosum* samples (see Figure 27 and Figure 28).

For the fermented puree samples of *V. myrtillus* and *V. corymbosum*, the same analytical procedures were applied. Optimal measurement results were obtained using 25 µL of *V. myrtillus* juice and 100 µL of *V. corymbosum* juice for both polyphenol and anthocyanin quantification.

Brix values determined with refractometer

The Brix value (°Brix) represents the concentration of soluble solids in a liquid and corresponds to approximately 1 gram of sucrose per 100 grams of solution. Although various soluble compounds like sugars, organic acids and amino acids contribute to this value, sugars are typically the most abundant soluble solid compound in fruit juices. Therefore, Brix values can be used as an indicator of the sugar levels in the berry samples analyzed in this experiment (Ahmed et al., 2022).

To determine the Brix values, a manual handheld refractometer (RHB-32ATC, Hong Han GmbH, 652 Germany) was used. A small volume of the berry juice was applied to the prism surface using a pipette. The instrument was held up against a light source and the value was read directly from the internal scale. All measurements were conducted at room temperature (approx. 22 °C).

Alcohol content

A standard laboratory-scale glass distillation apparatus, as depicted in Figure 31, was used to determine the alcohol content of the fermented berry juices. The water vapor distillation method used was adapted from the European Pharmacopoeia, 5th ed. (*Pharmacopoeia Europeae*, 2005). The setup consisted of a 250 mL round-bottom flask containing 25 mL of the juice sample and 100 mL of ddH_2O. 3 - 5 boiling stones were added before positioning the flask on a metal wire grid above a Bunsen burner. The flask was connected to a Liebig condenser via a distillation adapter. On the other side of the condenser, a 100 mL measuring flask was used as the receiving vessel for the distillate.

The alcohol content of the collected distillate was determined by measuring its density at a controlled temperature using a pycnometer. For this purpose, a clean and dry pycnometer was filled with the distillate, any excess liquid was carefully wiped from the top and weighed afterwards. The alcohol concentration was calculated based on the measured density using an online density-to-alcohol conversion tool (website: hobbybrennen.ch, last visited 28.10.2025). Each sample was distilled and analysed in duplicate.

Figure 31: Distillation apparatus set up

The ethanol concentration of all fermented berry juice samples was also calculated theoretically for comparative purposes, based on the final mass loss value after fermentation. As stated before, the conversion of glucose to ethanol and CO_2 follows the stoichiometric equation:

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH\ + 2\ CO_2$$
$$Glucose\ \rightarrow 2\ EtOH\ + 2\ CO_2$$

Based on this ratio, 1 mol of CO_2 (44.01 g) corresponds to the formation of 1 mol of ethanol (46.07 g). Using this relationship, the following formular was used to estimate the alcohol content by volume (v/v %):

$$Ethanol_{v/v}\% = \left(\frac{m_{CO_2} \times 1.047}{0.789 \times V_{sample}}\right) \times 100$$

mCO_2	Mass loss during fermentation (g), representing CO_2
1.047	Molar ratio factor between ethanol and CO_2
0.789	Density of ethanol (g/mL)
V_{sample}	Volume of fermented juice (mL)

4.6 General Information

Table 10: Tools and resources used to create the thesis

Tool/Resource	Purpose	Comments
ChatGPT (Open AI)	Language refinement and graph creation	Used to improve grammar, clarity and overall text flow Creation of Stacked-Area Plots (Figure 25, 26 and 27)
GraphPad Prism	Data analysis and graph creation	Used for generation of Figure 17 and 18 in chapter 4.4
Microsoft Excel	Data organization	Used to structure raw data, perform calculations, create table and charts
Microsoft Word	Thesis writing and formatting	Used for drafting, editing and formatting of the thesis
MolDraw	Chemical drawing	Used for generation of Figure 9 and 10 in chapter 2.1.3
SciFinder,PubChem, OpenEvidence	Research	Used for literature and compound research
Zotero	Reference Management	Used to organize literature, manage citations and generate bibliographies

AI declaration

AI tools/methodology was not used to create content or change content-related aspects of the thesis. The specific use of AI tools is described in Table 10. For grammar revision and clarity enhancement, ChatGPT-5.2 (https://chatgpt.com/) was used. Prompts such as “Please improve grammar and correct wording/fluency of the text.” were provided. The generated output was used to support the revision and grammar checking of my thesis. All AI-generated suggestions were carefully reviewed and proofread before inclusion.

4.7 Instruments and Lab Ware

Information about the instruments and lab ware utilized in the course of this work are outlined in Table 11.

Table 11: Instruments and Lab Ware

Device	Description
Analytical balance	BP210D, Sartorius
Balance	Sartorius LC4801P
Centrifuge	Function Line, Labofuge 400, Heraeus
Drying cabinet	Heraeus Instruments
Eppendorf Tubes	Eppendorf safe-lock tubes
Freeze dryer	VaCo 5-II D by Zirbus Technology, coupled with Vacuubrand Chemistry-HYBRID pump and equipped with flask drying rake
Grinder	Minigrinder, KYG
pH meter	HI223, Hanna Instruments
pH Test strips	MColorpHast™ pH-indicator (non-bleeding), EDM Millipore
Pipette	Eppendorf Research
Pipette Tips	Eppendorf, Sarstedt, StarLab
Refractometer	RHB-32ATC, Hong Han GmbH
Rotary Evaporator	Rotavapor R-210, Büchi
Sample Concentrator	Sample concentrator, FSC400D, Techne
Shaker	Orbital shaker 300S, GFL
SFC	ACQUITY UPC^2 by Waters. Modules: PDA, ELSD, QDa, ISM
SFC column	Torus 1-AA (1-Aminoanthracene), 130 Å, 1.7 µm, 3 x 100 mm
TLC capillaries	Micropipettes 1/2/3/4/5* µL, BLAUBRAND®intraMARK, BRAND®
TLC plates	TLC Silica gel 60 F254, Aluminium sheets 20 x 20 cm, Merck
TLC spray cabinet	CAMAG® TLC Spray Cabinet II
Ultrasonic Bath	Transsonic T 460, Elma
UPLC	ACQUITY UPLC H-Class System by Waters. Modules: PDA, QDa, ELSD, ISM
UPLC column	ACQUITY BEH C 18 (2.1 x 100 mm, 1.7 µm)
UV lamp	CAMAG®
UV-Vis Spectrometer	Shimadzu UV-1800
Vacuum pump	V-710, Büchi
Vortex	REAX 2000, Heidolph

4.8 Chemicals and Reagents

Information about the chemicals and solvents utilized in the course of this work are outlined in Table 12.

Table 12: Chemicals and reagents

Chemical/Reagent	Description
Acetone	Acetone Normapur, ÖAB distilled
Acetic acid	Merck (Darmstadt, Germany)
CC-6 polyamide	Carl Roth GmbH
Catechine	Sigma-Aldrich
Conc. Ammonia	Sigma-Aldrich
Chloroform	Chloroform Normapur, VWR chemicals
CYA-3-GLU	Sigma-Aldrich
ddH2O	Double-distilled water, purified by ion exchanger
Dichloromethane	Dichloromethane Normapur, VWR chemicals
Draggendorff reagent	Sigma-Aldrich
Ethanol 96%	ÖAB distilled
Ethyl acetate	Ethyl acetate Normapur, VWR chemicals
Folin-Ciocalteau reagent	Merck (Darmstadt, Germany)
Methanol	Methanol Normapur, VWR chemicals
Hexane	n-Hexane Normapur, VWR chemicals
Sodium carbonate	Merck (Darmstadt, Germany)
Sulfuric acid	95.0 – 98.0 %, ACS reagent, Sigma-Aldrich
Vanillin	99 %, RegaentPlus, Sigma-Aldrich

5 REFERENCES

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6 APPENDIX

6.1 Extracts

Table 13: Alkaloid extracts

Sample	Plant material Weight [g]	Extract weight [mg]
AT3	2,98323	39,30
AT5	2,01259	15,98
SK2	2,01020	5,52
CZ1	2,01844	8,70
NO1	2,00330	2,26
EE1	2,04419	2,53
AK2	2,00875	5,34
VM1	2,01077	5,44
AB	0,25061	1,80
LP	0,51797	4,27

Table 14: LLE extracts

Sample	Extract weight [mg]	Berry material weight [mg]
AT3	453,30	602,70
AT5	370,13	599,37
SK2	426,45	599,90
CZ1	375,32	601,87
NO1	298,87	599,90
EE1	346,16	599,98
AK2	414,88	604,20
VM1	365,34	603,15
AB	245,82	599,60

6.2 Figure and Table Legend

Figures

Tables