Using SIFT-MS for Detection of Beer Spoilage Bacteria and Compounds Causing Off-Flavour in Beer

A thesis presented to

The Faculty of Graduate Studies

of

Lakehead University

by

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In partial fulfillment of requirements

For the degree of Master of

Science in Biology

September 29 2014

Abstract

Selected ion flow tube mass spectrometry (SIFT-MS) was used for detection of spoilage bacteria in wort and beer. Samples were analysed in triplicate using SIFT-MS Full Scan mode to identify volatile compounds in the headspace of the samples. Volatile compounds in wort headspace included acetaldehyde, propanol, 2-methylpropanal, hexanal, methanethiol, 2-methylbutanal. Volatile compounds in the headspace of the wort inoculated with *Lactobacillus brevis* included ethanol, acetic acid and propanol. The following compounds were identified in the headspace of the sample of degassed beer: ethanol, propanol, 2-methyl-1-propanol, acetaldehydes, and ethyl formate. The detected volatile compounds coming from the headspace of wort inoculated with *Lactobacillus brevis* revealed changes in the compounds due to unwanted bacterial fermentation. In addition, an unidentified product ion having *m/z* 39 was detected. Calculations of relative abundance for this product ion and further investigations demonstrated that it can be considered as a result of compounds formed due to contamination with *L. brevis*. These data suggest that SIFT-MS has great potential for detection of volatile compounds coming from beer spoilage bacteria.

Lay Summary

The most famous gastronome of them all, Jean Anthelme Brillat-Savarin, wrote in "Physiologie du Gout, ou Meditations de Gastronomie Transcendante (1826): "Tell me what you eat and I will tell you what you are." And since we are what we eat, there is no room for error in the Food Processing Industry.

A new technique, called Selected Ion Flow Tube Mass Spectrometry (SIFT-MS), for detection of beer spoilage bacteria has been investigated. This novel method helps with early, rapid detection of beer spoilage bacteria and detects compounds that are responsible not only for the good aroma and taste of beer, but also has the potential of detecting compounds that can cause future off- flavours in final beer.

This study helps with improvement of the quality control process in breweries and can be considered as a new and faster method for detection and identification of compounds that can cause off-flavours in final beer.

Acknowledgment

I would like to express my appreciation to everyone who helped me with the progress of my Master‟s project thesis. Natural Science and Engineering Research council (NSERC) provided funding for the project and our industrial partner Sleeping Giant Brewing Co, helped with samples (beer and wort) for this project to be accomplished.

I would like to thank to my primary supervisor Dr. Lada Malek for giving me the opportunity to be one of his students and for all the help, guidance and support I received from him in the duration of my Master‟s degree. I would like to thank Dr. Brian Ross for his acceptance to be my co-supervisor, for the time he spend with me explaining me how to interpret the spectra from SIFT-MS and for all the support I got from him. I would like to thank Dr. Kam Leung for agreeing to be my committee member and for all the advices I got from him during committee member meetings.

I would like to thank to my colleague in the laboratory Dr. Andrew Gibson for the help with SIFT-MS software and motivation.

I am grateful to my husband Vasil Georgiev for all the support, enthusiasm and motivation that helped me to achieve my goal.

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1. Introduction

The purpose of this study is to investigate the potential usage of Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) in the process of beer production. The main goal is detection of volatile compounds in wort and beer and describes an investigation of the effect of *Lactobacillus brevis* on wort and final beer. Sweet liquid called wort, usually contains aldehydes, in varying amounts [1]. If certain aldehydes are present in high quantities this could cause off-flavour in the final beer. Although, beer as a final product is microbiologically stable it still can get contaminated due to improper hygiene, contamination caused by raw materials, airborne contaminants, dispensing equipment etc. [2]. The problem with beer spoilage caused by bacteria is typical not only for microbreweries, but also for major breweries [3, 4]. The most common beer spoilage bacteria are *Lactobacillus brevis*. Thirty five percent of all cases of spoiled beer are due to these bacteria [5]. For that reason the bacterium used in this study is *Lactobacillus brevis* (ATCC 4006) grown in selective de Man, Rogosa, and Sharpe (MRS) media.

1.1 Background and literature review

1.1.1 Beer production

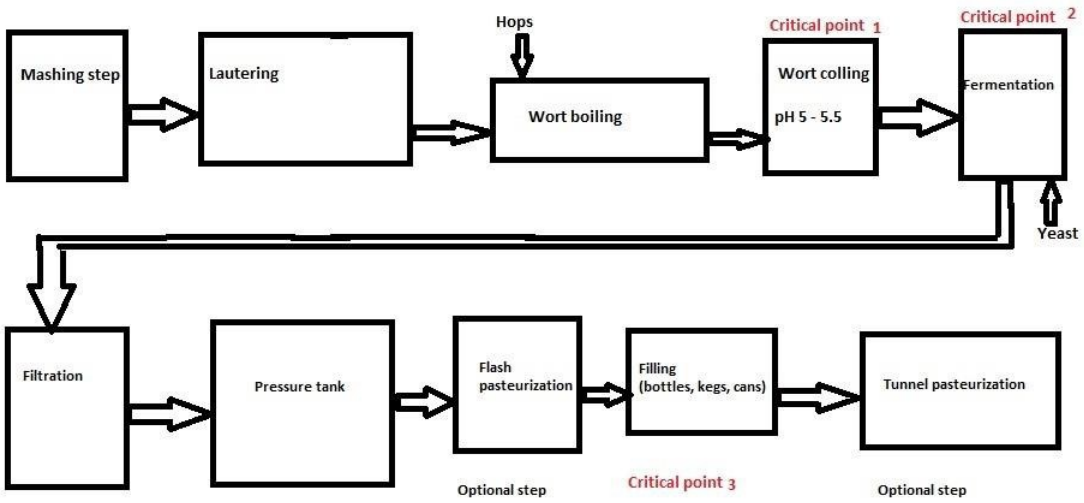

Figure 1. Production line of beer with critical control points

Beer brewing consists of several steps (Fig. 1). Malt is prepared from grain, most commonly barley, which is allowed to germinate to convert starch into maltose. The malted grain is dried or even roasted. Malt is extremely important for the quality and taste of beer. Usually malt is bought by the modern breweries and it is not something they do as part of the brewing process. First malt is ground to break apart kernels and increase surface area. Next the malt is soaking in water and let it sit for about an hour. This is called the mashing step. During this step the malt enzymes break down the starch into fermentable sugars, resulting in very sweet liquid called “wort”. During “lautering”, the

wort is separated from husks and other particles by a perforated screen inside a vessel called lauter tun that retains solids and allows wort to flow through [6, 7]. The liquid wort is transferred to a brew kettle, where it is heated to vigorous boil continuously for an hour. During this step hops are added and sometimes additional sugars. The boiling process terminates enzymatic processes, precipitates proteins, and isomerizes hop resins. The "hopped" hot wort goes through heat exchanger where it is cooled to between 1^{o} and 21^{o}C and aerated before yeast can be introduced for fermentation in the fermentator. The temperature to which the wort must be cooled down depends on what type of beer is going to be produced – ale or lager. Ales are fermented at temperatures 15^{o} to 21^{o} C for one to three weeks. This higher temperature allows an increase in yeast produced flavours. Lager beers are fermented at temperatures 1^{o} to 10^{o} C for one to three months. At these lower temperatures, the yeast needs longer time to complete fermentation and produces fewer flavour compounds. After the yeast metabolizes all of the available food, producing alcohol and carbon dioxide, it goes dormant and collects on the bottom of the fermenter. Some beers are filtered after fermentation and then transferred into cold pressure tank, where they are carbonated and ready to be bottled or kegged.

1.1.1.1 The role of hops in beer

Evidence exists for beer being produced around 6000 BC, but hops began to be used in beer production only about 1000AD [8]. It was found that hops possess specific properties that are beneficial for beer and keep the drink without off-flavours for a longer time. In 1516 AD this resulted in a guild of Bavarian brewers creating the

Reinheitsgeobot purity law for beer production, which stated that it is illegal to use any other ingredients than water, barley and hops (at this time people did not know of the existence of yeast) [8]. Back then it was believed that hops will prevent beer from spoiling. However, it was found out later that hops inhibit growth of Gram positive bacteria only [9].

1.1.1.2 Hop plant

The hop plant *Humulus lupulus* L., is a vine from the hemp family. It is dioecious and blooms yearly. Only cones, the female flowers, are used in beer production. The most important part of the flower for the bitterness and preservation of beer are golden resinous granules, at the base of floral bracts. Detailed analysis of hop resins identifies a number of compounds (Fig. 2).

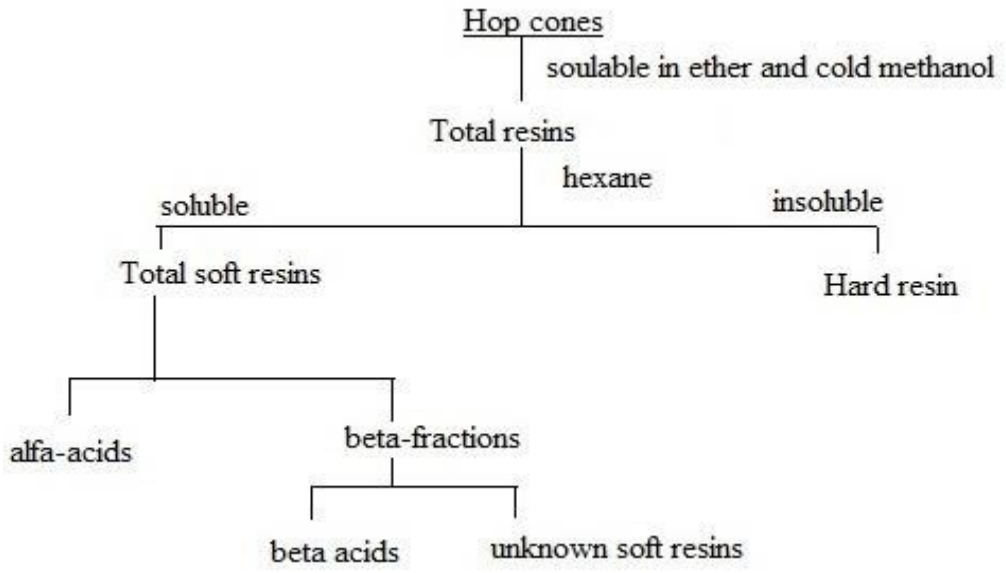

Figure 2. Hop extraction and fractionation (modified from [10])

The α-acid is a mixture of homologous compounds. During the wort boiling stage α-acid rearranges to form *iso*-α-acid compounds, which are much more bitter and soluble than the α-acid. The α-acid fraction is a mixture of three main compounds: humulone, cohumulone and adhumulone. The bittering compounds of beer contain three analogues of these α-acid known as *iso*-α-acids: isohumulone, isocohumulone and isoadhumulone [9]. The isomerization rate of α-acids is low, typically of the order of 30 %, due to relatively acidic condition of wort (pH 5.2) and the adsorption to the wort coagulum during boiling and fermentation [9, 11]. β-acids or lupulones in hops cannot undergo the same process of isomerisation as α-acids due to their poor solubility in wort and beer. Thus, they are not transferred into beer and have no direct value in brewing process [9, 11].

The antibacterial activities in α-acids and β-acids are higher than *iso*-α-acids, but they dissolve to a lesser extent in beer and water. The antiseptic properties of hopped wort are related to Gram positive bacteria [12, 13]. The antiseptic strength of hops increases in lower pH solutions and is associated with permeability changes of bacterial cell wall [9, 13]. The hops composition was found to cause leakage of the cytoplasmic membrane of *Bacillus subtilis*, which results in inhibition of transport of sugar and amino acids. Afterward, inhibition of respiration and synthesis of protein, RNA and DNA have been also observed [9, 14]. Hop compounds are weak acids and the undissociated forms at wort pH are mainly responsible for inhibition of bacterial growth.

1.1.2 Beer spoilage bacteria

1.1.2.1 Hop resistance of lactic acid bacteria

Antiseptic properties of hops are related to Gram positive bacteria. However, there are certain species, such as *Lactobacillus* sp. and *Pediococcus* sp. that are resistant to hop compounds and can cause beer spoilage. The Gram positive *Lactobacillus brevis* is considered one of the most resistant species to hop compounds. The resistance of these bacteria to hops varies from strain to strain and also can be related to the prolonged subculturing of *L. brevis* in absence of hops [12, 13]. On the other hand, subculturing *L. brevis* in media containing hop increases its resistance to hops 8 to 20 times [9, 15].

Several mechanisms of hop resistance by lactic acid bacteria have been proposed: enzymatic drug inactivation, target alteration, inhibition of drug influx, active extrusion of drugs etc. These alternatives are described in details as follows:

The enzymatic drug inactivation occurs when bacteria become resistant to an antibacterial agent by producing enzyme that eliminates the antibiotic. However, no conversion or inactivation of *trans*-isohumulone has been observed in hop resistant strains of *L. brevis* [16]. Hence, it is not known how *L. brevis* resists hops compounds.

In target alteration mechanism, cellular targets of antibiotics can be changed due to mutation or enzymatic modification and cause resistance of bacteria. Although the target is the cell membrane in the case of trans-isohumulone [17, 18] it has not been investigated enough whether hop resistant strains of *L. brevis* have changed lipid or

protein composition of their membrane to lower the absorption of hop compounds. That is why this mechanism cannot be considered as the one responsible for *L. brevis* resistance.

Inhibition of drug influx mechanism relates to the observation that the cell walls of Gram positive mycobacteria have been found to be excellent barriers for lipophilic drugs. The infusion of hop compounds might also be affected by the existence of a galactosylated glycerol teichoic acid in beer spoilage lactic acid bacteria [19].

An overexpressed multidrug resistance pump HorA has been found in some strains of *L. brevis*, when they are exposed to hop compounds. This accelerates active extrusion of the drug. Also, in some strains a proton motive force-dependent hop excretion transporter was suggested [20, 21, 22]. This could explain some of the lactic acid bacteria resistance to hops compounds.

The mechanism responsible for *L. brevis* hops resistance is illustrated in Fig.3. Acting as ionophores, hop compounds exchange protons for cellular divalent cations. In a cell that is sensitive to hops, hop compounds (Hop-H) invade the cell (due to higher internal pH) and dissociate into hop anions and protons. Hop anions trap divalent cations such as Mn^{2+} and diffuse out of the cell. The diffusion of hop-metal complex with ionophoric action results in an electroneutral exchange of cations. Intracellular pH is decreased due to the release of protons from hop compounds. Also, the release of protons results in a dissipation of the transmembrane proton gradient (DpH) and the proton motive force (pmf). As a consequence, pmf-driven uptake of nutrients will be decreased. In hop-resistant cells, hop compounds can be evicted from the cytoplasmic membrane by

HorA(a) [20] and probably also by a pmf-dependent transporter (b) [22]. Furthermore, overexpressed H^+-ATPase increases the pumping of protons released from the hop compounds (c) [20]. The ATP generated from hop-resistant cells is more than the one in hop-sensitive cells [16]. Galactosylated glycerol teichoic acid in the cell wall [19] and a changed lipid composition of the cytoplasmic membrane of beer spoilage lactic acid bacteria may increase the barrier to hop compounds.

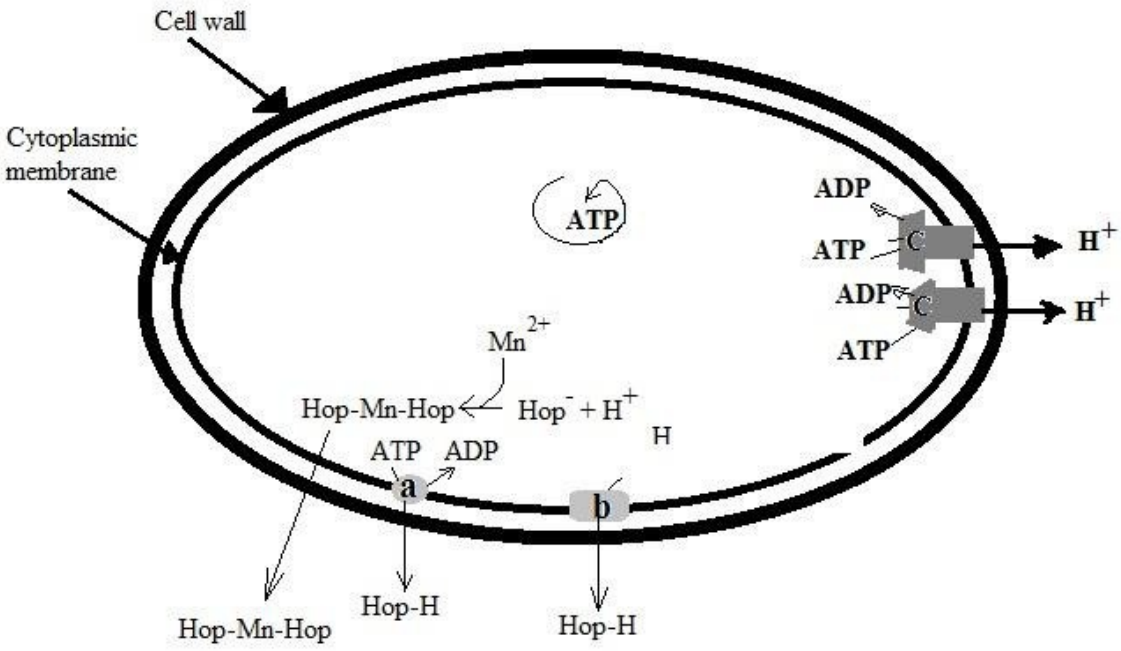

Figure 3. Mechanism of hop resistance (modified from [9])

1.1.2.2 Bacterial contamination of beer

In comparison to water beer has strong microbiological stability. The high levels of ethanol (ranging from 0.5% - 10% alcohol by volume, but usually 4 – 5 % in commercial beers) [23], antiseptic action of hop compounds and lower pH in beer make

the environment adverse for bacterial growth [24, 25]. Until now no human pathogen has been found to survive in alcoholic beer, but the situation can be different for non – alcoholic and unpasteurized beer [26]. In spite of stability and unfavourable environment in beer, there are certain organisms that can cause beer spoilage during the production process. Most of these bacteria are Gram positive (particularly *Lactobacillus* spp.) and only few are Gram negative bacteria. Contaminations with these bacteria can occur during the preparation of beer in the brewery. Spoilage bacteria can be present in raw materials and can cause contamination of wort, retard fermentation, or may cause deterioration of final beer [27]. Some of these bacteria are presented in Table 1 with the volatile compounds they produce.

Table 1. Effect of some contaminations on the fermentation and on final beer. Spoilage organism is in the first column, second column explains if this organism has an effect on fermentation. Bacteria that cause turbidity has a sign "+" and those that do not cause this effect has "-". Last column describes compounds these bacteria produce in final beer that cause off-flavour. (Modified from reference [2])

Group or genera	Effects on fermentation	Turbidity	Compounds in final beer causing off-flavour
Wild yeasts	Super-attenuation	+	Esters, fusel alcohols, diacetyl, phenolic compounds, H_2S
Lactobacilus, Pediococcus		+	Lactic and acetic acids, diacetyl, acetoin
Acetobacter, Gluconobacter		+[1)]	Acetic acid
Enterobacteria	Decreased fermentation rate, formation of ATNC	-	DMS, acetaldehyde, fusel alcohols, VDK, acetic acid, phenolic compounds
Zymomonas		+[2)]	H_2S, acetaldehyde

Pectinatus	+	H_2S, methyl mercaptane, propionic, acetic, lactic and succinic acids, acetoin
Megasphaera	+	H_2S, butyric,cvaleric, caproic and acetic acids, acetoin
Selenomonas	+	Acetic, lactic and propionic acids
Zymophilus	+ [3)]	Acetic and propionic acids
Clostridium	-	Butyric, caproic, propionic, and valeric acids

ATNC - apparent total n-nitroso compounds; DMS – dimethyl sulphide; VDK – vicinal diketones, Fusel alcohols; n-propanol, iso-pentanol, iso-amylalcohol; 1) In the presence of oxygen; 2) in primed beer; 3) at elevated pH (5-6)

When looking at the production line of beer (Fig. 1), one can find a number of critical control points where contamination can occur. Furthermore, in today"s production of beer most of microbreweries do not use pasteurization (optional step in Fig. 1) and, in many breweries, hygiene is poor [3, 25]. This creates an opportunity for spoilage of bottled beer due to anaerobic bacteria. The contaminations originating from yeast, wort and fermentation will have serious consequences for the product and will cause off-flavour in the final beer [9, 24, 25].

In the case of insufficient hygiene bacterial growth can begin and have an utmost impact on the process of beer making [26]. The first possible step where contamination can occur is cooling of wort. Wort liquid has a pH 5 – 5.5 and high nutrient content, which favours development of bacteria in sweet wort. Some beer spoilage bacteria like lactic acid bacteria can cause contamination from wort until final beer [9]. The most common beer spoilage bacteria from genus *Lactobacillus* are *Lactobacillus brevis* and

Lactobacillus lindneri. The most common beer spoilage organisms from *Pediococcus* genus are *Pediococcus damnosus* and *Pediococcus inopinatus*.

L. brevis is responsible for 35% of known beer spoilage cases in breweries [5]. The reason for the high percentage of spoiling is that *L. brevis* is an obligate heterofermentative bacterium. Also, it will grow at optimal temperature of 30^{o} C and pH 4 – 6. This species grows equally well on media in laboratory and in beer [5], and this is probably the reason for their predominance in incidents of beer spoilage [27].

L. lindneri is responsible for 25% of beer spoiling cases in breweries. This bacterium is highly resistant to hop compounds, grows optimally at 19^{o} – 23^{o} C. All strains of *L. lindneri* are capable of spoiling beer. This bacterium is difficult to grow in laboratory, but grows fast in beer [5, 27]. Moreover, it has been reported that *L. lindneri* can survive even through optimal pasteurization process [28].

Pediococcus damnosus is highly hop resistant, tolerates low pH and can grow in high ethanol, where *P. inopinatus* can grow at pH higher than 4.2, low concentrations of ethanol and hop compounds [5].

So far it was considered that only high hop resistance and low pH tolerance are responsible for growth of *Lactobacillus* sp. in beer. However, recent research suggested that there is a connection between hop sensitivity of bacteria in presence of ethanol [29]. According to this research some *Lactobacilli* that are usually sensitive to hop compounds will become more resistant in presence of ethanol. Each bacteria mentioned above, once growing in beer, will cause off flavour in the final product due to the formation of lactic

and acetic acids, and to diacetyl. Diacetyl (2, 3-butanedione) is part of class compounds vicinal diketones (VDK). It has a four carbon chain and two ketone groups are located on the second and third carbons of the chain. Diacetyl causes a buttery to butterscotch taste in beer. The threshold for diacetyl in beer is 10 ppb to 40 ppb [30]. Once bacteria start to grow in the wort, they will be transferred to the fermentation tank. During fermentation diacetyl is continuously produced. When fermentation is finished, the yeast will take diacetyl into their cells and reduce it through enzymatic activity to acetoin and 2, 3-butanediol. Acetoin (or 3-hydroxybutanone) and 2, 3 – butanedione give a buttery taste to beer [31, 32] which is considered as an off-flavour in final beer.

As well as producing off taste due to ability of *Lactobacillus* sp. to ferment starch and dextrin, they can cause super-attenuation [9, 33]. As wort or beer is fermented by culture yeast, the lowest specific gravity it can reach is addressed as its attenuation limit. In the brewing process, the attenuation limit is important, because subtracting it from the present specific gravity of the beer gives Residual Fermentable Matter (RFM). RFM is essential especially for naturally conditioned beer and influences the rate of conditioning and pressure in the bottle. If RFM is too high the beer will come to condition rapidly and could reach the state of over-condition, which can cause leaks from casks. Then again, if RFM is low then beer could remain flat. In cases when only pure culturable yeast is used, specific gravity of beer falls to the attenuation limit and then remains constant. However, there are certain types of beers like old English Stock and export beers that depend on the secondary fermentation where they use yeast from genus *Brettanotnyces*. This secondary

fermentation has a definite limit. The case when the gravity of beer is below this secondary fermentation limit is referred as super-attenuation [9, 33].

1.1.3 Detection methods available

Various methods for detection of beer spoilage bacteria have been developed. So far, for each one of them enrichment of the culture is needed. Beer spoilage lactic acid bacteria (LAB) are not pathogenic to humans and thus there are no regulations from the authorities. However, they cause significant losses for breweries by retarding fermentation, causing off-flavour of semi-final and final beer, required extensive cleaning procedures. That is the reason for European Brewing Convention (EBC), American Society of Brewing Chemists (ASBC) and the Brewery Convention of Japan (BCOJ) to approve methods to be used for detection of beer spoilage organisms. For LAB detection limit should be 0 – 50 CFU (colony-forming units) in 100 – 250 mL sample volume. In pitching yeast a single spoilage organism should be detected in 10^6-10^8 cultivation yeast cells. That is the reason the only method approved by EBC, ASBC and BCOJ (from the mentioned above) for detection of beer spoilage bacteria is cultivation method [27].

There are number of methods available for detection of spoilage bacteria. They include: cultivation based method, polymerase chain reaction (PCR), fluorescence microscopy, and HybriScan [34].

1.1.3.1 Cultivation based method

Cultivation based method includes selective pre-enrichment of the sample with microscopic read out. The time needed for this method is 3 – 7 days. The advantages of this method are that is highly sensitive (detection limit as low as 1CFU) and can be used for detection and identification of all beer spoilage bacteria. The disadvantages are that is time consuming; it cannot detect non-culturable microbes (important for *L. lindneri* that is hard to culture in laboratory conditions but grows fast in beer [28]) and is labour intensive, and it is expensive.

1.1.3.2 Polymerase chain reaction (PCR)

PCR and real-time PCR method can be used for identification of all relevant beer spoilage organisms. PCR is based on assay for spoilage associated genetic markers. For example, for *L. brevis* proposed beer-spoilage marker genes are *hit*A, *hor*A and *hor*C [35]. The sample preparation needed for this method includes enrichment and lysis of bacteria, also, if necessary, pre-enrichment of the sample. Time needed for the PCR method varies from 3 hours to 2 days. The detection limit is 1 – 5x 10^3 CFU. The advantages of this method are that it has high sensitivity and produces quantitative analysis. The disadvantage is that an expensive device is needed.

1.1.3.3 Fluorescence microscopy

Fluorescence microscopy can be used for detection of *Lactobacillus* spp. and *Pediococcus damnosus*, *Lactobacillus* spp. + *L. brevis*, *Pectinatus* spp. + *Megasphaera*

cerevisiae. Sample preparation for this method include pre-enrichment. The time needed for the VIT method is 2 days. The detection limit is $1x10^3$ CFU. The advantages of this method are: simple detection technology set up and detection of only living cells (RNA). The disadvantages for this method are that it is time consuming, expensive, needs a fluorescence microscope, it is not automatable, and it is difficult to analyse the data.

1.1.3.4 HybriScan

The HybriScan method is based on the detection of rRNA via hybridization events and specific capture and detection probes [34]. It targets conserved or unique rRNA sequences. It uses labeled capture probes to immobilize the target sequence on a solid support plate, then a labeled detection probe provides an enzyme-linked optical signal read out. Detection is achieved by antibody labeled enzymes. Results can be seen by chromogenic substrates. This method can be used for the identification of all relevant beer spoilage microorganisms. Sample preparation includes enrichment and lysis of bacteria, and if necessary pre-enrichment of the culture. The time needed for this detection method varies from 3 hours to 2 days and the detection limits are $1\text{-}5x10^3$ CFU. The device needed to perform this method is microplate reader. The advantage of this method is that it performs quantitative and qualitative detection of living cells. The disadvantages are that it has no differentiation of stereotypes or subspecies and it has limited probe design (rRNA target).

1.1.4 Wort – quality and deterioration

When it comes to the quality of beer we look at certain criteria: foam, stability, colour, clarity, biological stability, physicochemical stability and most importantly taste and aroma profile. Beer flavour comes primarily from hop compounds (bitter acids), ethanol, and carbon dioxide. However, there are some unfavourable aspects of beer flavour quality particularly resulting from the instability of final beer product. This includes reduction of favourable flavour compounds, like bittering from hops and emergence of aging flavour compounds (sweet, cherry, cardboard tastes) [36]. The off-flavours caused by malt and wort compounds are different than ones caused by beer spoilage bacteria. Flavour stability is still difficult to control due to the complexity of malt and beer production, the composition of beer, and also the variety of compounds that influence beer flavour stability. An important factor in beer aging is the increase of aldehyde concentration. Higher aldehyde concentration leads to negative effects on aroma, taste and mouthfeel [37]. Aldehydes used as markers are Strecker aldehydes, linear fatty acid aldehydes and Maillard reaction products (Table 2 from Ref. [37]).

Table 2. Aldehydes that cause off flavour in beer over time (from [37]).

	Aldehydes	Flavour description
Strecker aldehydes	2-Methylpropanal	Grainy, varnish, fruity
	2-Methylbutanal	Almond, apple-like, malty
	3-Methylbutanal	Malty, cherry, almond, chocolate
	Methional	Cooked potatoes, worty
	Phenylacetaldehyde	Hyacinth, flowery, roses
Linear fatty acid aldehydes	Hexanal	Green, grassy, winey
	trans-2-Nonenal	Cardboard, papery, cucumber
Maillard reaction product	Furfural	Caramel, bready, cooked meat

Each of these aldehydes could exist in malt. The taste threshold is different for each of these aldehydes and is an important factor for beer instability. Once the wort is boiled and hops and sugars are added it is important to investigate the volatile compounds coming from the boiled wort because it is important to know if the wort is clear from aldehydes or bacteria that can ultimately cause spoilage or off flavour during aging of the beer.

1.1.5 SIFT–MS

As described in 1.1.3 current methods for detection of beer spoilage bacteria are not ideal. Most of them are time consuming and/or not sensitive enough. Furthermore, all of these methods rely on detection of bacteria. However, in case of deterioration of wort

there are volatile compounds produced (Table 2) that can cause off-taste in final beer. Therefore, it would be beneficial to look for a method for detection of spoilage organisms that can detect these volatile compounds responsible for off-flavours in final beer that could give faster results.

Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) is an analytical technique for the investigation of traces of gases and volatile organic compounds. This technique could be ideal for breweries, where early determination of gaseous off-flavour producing bacterial contamination (Table 1) and aldehyde content (Table 2) is crucial for beer quality. Moreover, it can perform flavouring analysis [38] and could be used as a quality control method to monitor fermentation, to look out for bacterial contamination and to confirm that the brew is developing as expected.

1.1.5.1 Background

The selected ion flow tube mass spectrometry (SIFT-MS) detection and analyses of gases has been realized through a research developed by David Smith and collaborators [39]. The applications for SIFT-MS quickly expanded and already includes breath analysis [40, 41], environmental monitoring [42], oil exploration [43], ambient air monitoring for occupational safety and health [44], the detection of chemical warfare agents [45], peroxide-based explosives [46], and most relevant to this work, volatile malt aldehydes [47].

1.1.5.2 Basic principles involved in SIFT-MS

SIFT-MS is a „soft ionisation" technique in which charged precursor ions are reacted with trace gases to produce distinctive product ions at a known rate. The precursor ions are positively charged (H_3O^+, NO^+ and O_2^+) and are formed by the microwave excitation of low pressure water vapour (Fig. 4).

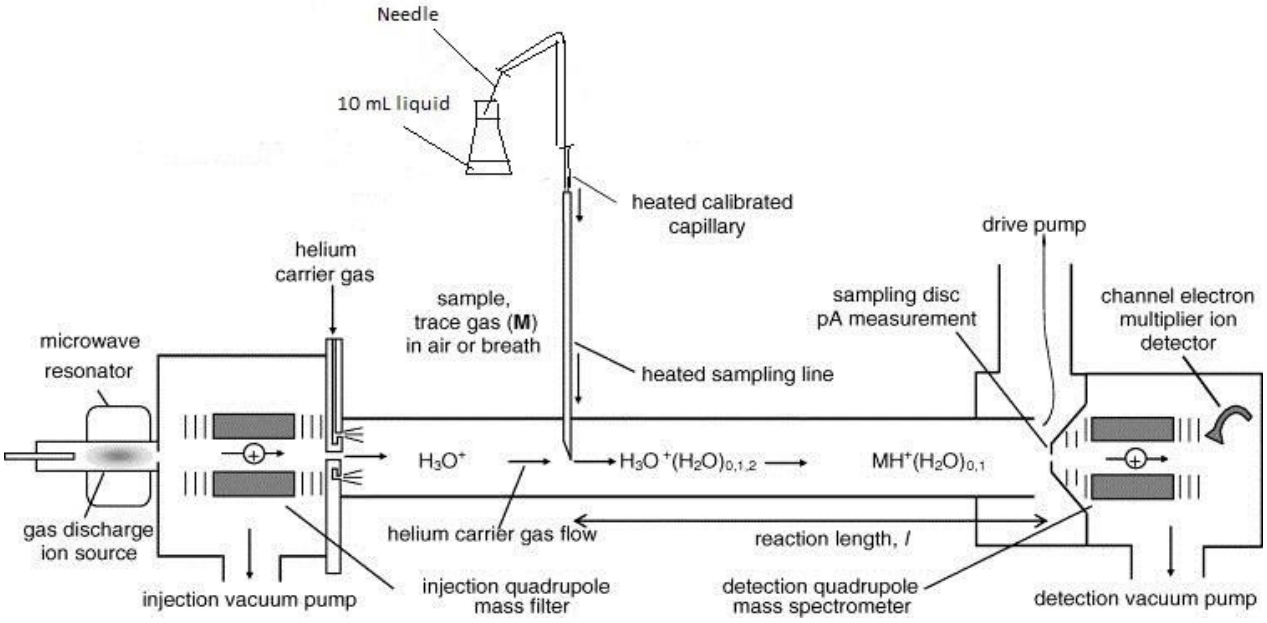

Figure 4. SIFT-MS diagram with indicated precursors, carrier gases and way of operation (From references [48, 49]).

The precursor ions (H_3O^+, NO^+ and O_2^+) are selected using a quadrupole mass filter and are injected into the flowing helium carrier gas passing through a flow tube where they react with the sample gas flow. The resulting product ions are detected and quantified using a downstream mass spectrometer/ion multiplier combination. The precursor ions do not react with components of air, like nitrogen, oxygen, water, argon and carbon dioxide, but they will react with many other volatile trace compounds [50].

Given that this reaction occurs at a known rate which can be experimentally determined, the rate of product ion production can be used to calculate the absolute concentration of analyte, thereby, avoiding the need for calibration standards. This added to technical simplicity, high sensitivity (approximately 1 ppb) and real time analysis makes SIFT-MS an ideal tool for the investigation of head space gases.

For head space analysis the gases are introduced in the SIFT-MS using negative pressure via needle piercing cap foil containing 10ml liquid in calibrated capillary [51]. The sample is introduced into flow tube (downstream at a flow rate approximately 0.3 Torr L sec^{-1}), where it reacts with selected ion precursors (dark rectangles on the left of Figure 4) [52]. The precursor ion is selected to best suit the product that is analyzed. An incorrect precursor will produce a reaction coefficient that is too slow or may be responsible for confusing *m/z* pattern. Poor chemical resolution is the major disadvantage of the technique, since different compounds, isomer or isobaric to one another, can react with the precursor ions to produce the same product ions. Fragmentation is another reaction happening, when the radical cations (M^{+}•) are unstable they will be fragmented into smaller ions. However the availability of three precursor ions frequently allows for compounds to be differentiated since unique product ions may be produced using at least one of the precursors [53]. The reaction between the sample and the second or the third precursors can provide information that confirms or rejects it from probable compounds. The possibility of using different precursors for chemical ionization allows for detection of otherwise indistinguishable compounds [54].The potential products of the reactions are

presented in Table 3. However, some volatile compounds do not follow the pattern presented below, when reacting with a certain precursor.

Table 3. Multiple precursor ion characteristics and their typical reactions, where M stands for molecular mass of reactant gas (from reference [37]).

Precursor	Flight tube		Detection
H_3O^+	Proton transfer	MH^+	M+1
NO^+	Hydride abstraction	$[M-H]^+$	M-1
	Hydroxide abstraction	$[M-OH]^+$	M-17
	Addition	$M.NO^+$	M+30
O_2^+	Charge exchange	M^+	M

The mass detector counts are amplified and reported via two reporting modes. In full scan mode a spectrum is produced (Fig. 5).

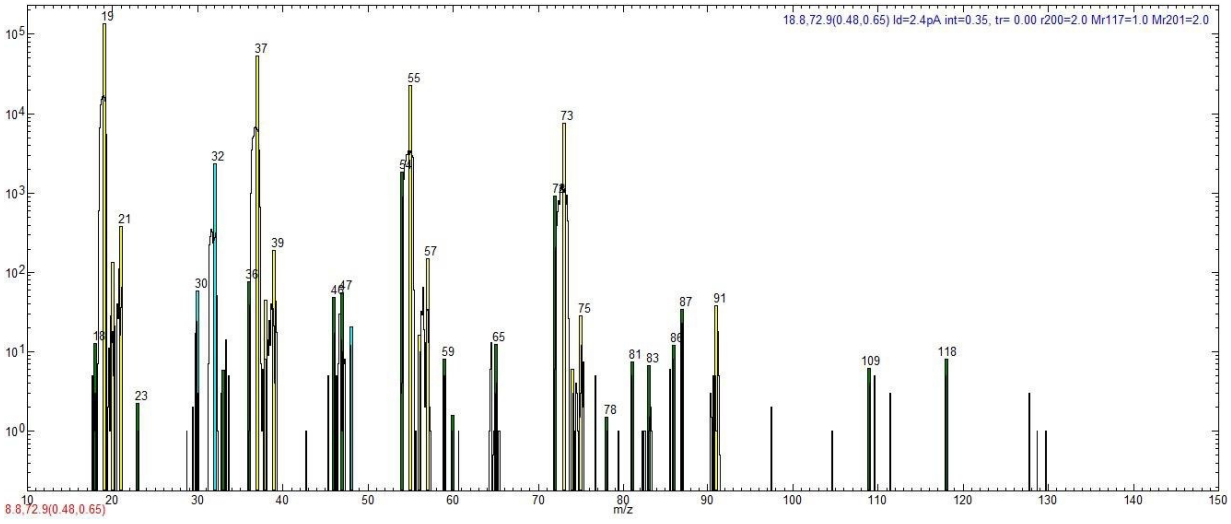

Figure 5. Example of water full scans of mass/charge ratio (*m/z*) against frequency of counts produced by SIFT-MS software.

The other counting mode used by SIFT-MS is in multi-ion-monitoring (MIM) mode, where ion intensities are recorded over specific *m/z* values and the mass spectrometer detects specific precursors and only selected specific products of the reaction. In this mode volatile gases can be detected and quantified faster (Fig.6) [51].

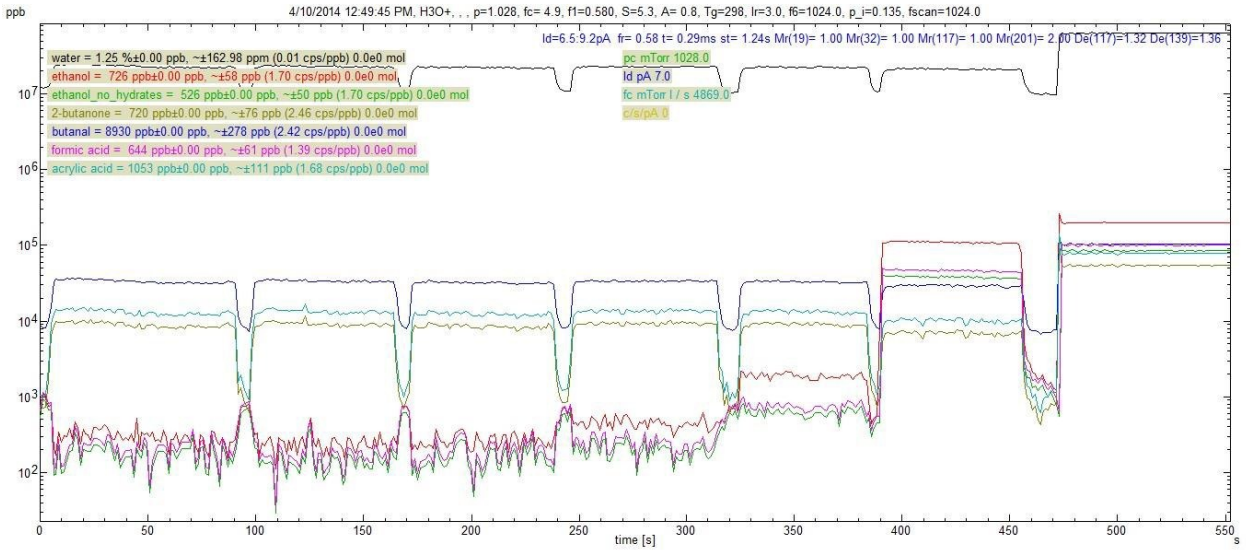

Figure 6. Example of MIM mode for detection of ethanol using H_3O^+ precursor ion. The plateaus represent calculated average concentrations (in ppb) of various (colour coded in the software) product ions.

The major difference between MIM mode and FS mode is that MIM represents product counts versus time, whether FS mode is related to product counts to *m/z*.

1.2 Objectives

SIFT-MS technique has a great potential for detection of contaminants and monitor flavours. Hence the specific objectives targeted by this work are the following:

1. To examine volatile compounds in wort headspace using SIFT-MS.
2. To examine volatile compounds coming from beer headspace using SIFT-MS.
3. To examine the possibility for early detection of *Lactobacillus brevis* inoculated in wort and beer using SIFT-MS.

2. SIFT-MS as a method for determination of volatile compounds coming from wort and wort inoculated with *Lactobacillus* sp.

2.1 Abstract

Selected Ion Flow Mass Spectrometry technique was used in this study to investigate volatile compounds coming from the head space of wort sample inoculated with *Lactobacillus brevis*. Two types of samples were investigated: one set was inoculated with one hundred microliters *Lactobacillus brevis* MRS broth, while the control set was plain wort and volatile compounds coming from it were identified. Each of the samples was measured on day 3, 7 and 14 after the inoculation. Volatile compounds produced as a result of *L. brevis* were ethanol and acetic acid.

2.2 Introduction

The sweet liquid that is prepared from soaked malt, with added additional sugars and hops is called wort. Wort is the starting material in the brewing process. After the wort is boiled and cooled it is transferred to a fermenter, where the yeast is added. However, if microorganisms contaminated the wort, then there are may be two outcomes: (1) the microorganism might have a positive influence on beer flavour [55] or (2) might negatively affect the fermentation (by retarding it or start production of diacetyl) [2, 31, 32]. For example, for Lambic fermentation (spontaneous fermentation) used today in parts of Belgium to prepare sour beer, once the wort is boiled it is exposed to open air overnight and later on is transferred and sealed in wooden barrels and stored at temperatures 0^{o} to 25^{o}C without further inoculation with yeast. Wooden barrels allow a

small amount of oxygen to enter during fermentation. Microorganisms involved in Lambic fermentation are *Saccharomyces cerevisiae*, *Enterobacteriaceae*, *Kloeckera apiculata*, *Lactobacillus* spp. or *Pediococcus* spp., *Brettanomyces bruxellensis and Br. lambicus*. Some of these microorganisms are pathogenic, but they do not survive once the yeast starts producing ethanol [55]. The presence of lactic acid bacteria species is interesting, because they are responsible for high percentage of beer spoilage. During the preparation process of Lambic beer *Lactobacillus* spp. *or Pediococcus* spp. are added when the yeast starts to die and they give the specific sour taste of this type of beer. However, the same species (*Lactobacillus* spp. *or Pediococcus* spp.) once added in the fermenter in anaerobic environment, might start producing diacetyl during fermentation and be responsible for buttery off-flavour in final beer [2, 32]. In both variations of bacterial influence in the process of fermentation, it is important to monitor the volatile compounds originating from fermented wort.

The SIFT-MS technique works as a chemical ionisation for detection of volatile compounds using three precursor ions (H_3O^+, NO^+ and O_2^+). The product of reaction of the volatile compounds and ions is detected. SIFT-MS has a great potential for detection of beer/ wort spoilage bacteria and wild yeast, because each one of them creates volatile compounds which can be detected in the early stages of the process of contamination. It is a well-known fact that *L. brevis* is one of the most common beer spoilage bacteria [5], but also plays an important role in production of Lambic beer [55]. The goal of this study is to investigate the effect of *L. brevis* on wort and the possibility detecting volatile metabolites produced by the bacteria in the wort headspace using SIFT-MS. Expectations

are that the bacteria will ferment the wort, but it is important to know to what extent. Also, this work is intended to demonstrate the possibility of using this technique for detection of volatile compounds originating from wort and follow their changes over time course of day 3, 7 and 14.

2.3 Methods and materials

2.3.1 Bacterial strain: *Lactobacillus brevis* (ATCC 4006) was used in this study as model beer spoilage bacterium. The bacterial culture was started from pellets rehydrated with 1 mL de Man, Rogosa, Sharpe (MRS) Broth. Aseptically rehydrated pellets were transferred into 40 mL liquid MRS Broth. Several drops from this suspension were used to inoculate a second flask of broth. Flasks were incubated at room temperature for 48 hours. After 48 hours the growth was evident by turbidity in the broth. From the inoculated tubes glycerol stock was prepared. Bacterial cultures used for this study were always started from frozen stock.

2.3.2 Glycerol stock for *Lactobacillus brevis*: Eighty percent glycerol with 20% water was autoclaved for 15 min. Then 200 µL of autoclaved 80% glycerol was added to 800 µL of *L. brevis* mixture pregrown in MRS Broth for 24 hours. The mixture was briefly vortexed and stored at -80^{o}C.

2.3.3 Growing the bacteria from frozen stock: The frozen stock was scraped using sterile pipette tip and streaked onto fresh MRS agar plates. The agar plates were incubated for 48 hours at 20^{o}± 2^{o}C. Single colonies were evident in the second and third section of the MRS plate after 48 hours. Using a sterile loop one colony was picked up

and inoculated in 40 mL liquid MRS media. After 48 hours bacteria were growing and turbidity was present in the broth.

2.3.4 Spectrometry: A GE-Healthcare Ultrospec 2100 pro UV/Visible Spectrophotometer was used to measure the absorbance of inoculated media at 600 nm. Fresh medium was used as a blank. For the purpose of this study cultures with optical density (OD) 1.0 were used. Day after inoculation of the media OD was measured. If the OD was higher than needed it was adjusted by adding media to the inoculated sample. Typical sample concentration process of adjustment follows.

40 mL media was inoculated with 1 mL liquid culture. After 24 hours the OD was 1.38.

C_1= 1.38, V_1 = X mL, C_2 = 1, V_2 = 39

So: $C_1 . V_1 = C_2 . V_2$

i.e. 1.38. X = 39. 1

X = 39/ 1.38 = 28.2 mL is the volume that needs to be used to dilute 38 mL to correspond to A_{600}=1.

Volume used to dilute to 38mL is 28.2 mL

Final absorbance was verified to be 1±0.02.

To ensure that equal amount of bacteria were inoculated into experimental media (wort), serial 10 fold dilutions were prepared and plated on agar plates, giving about 8.2 x 10^8 CFU per mL of A_{600}=1 suspension.

2.3.5 Wort: Freshly prepared wort with added hops and honey was obtained from Sleeping Giant Brewery, Thunder Bay. Hot wort was poured in sterile containers and later on autoclaved for 15 min at 121^{o}C in and stored in refrigerator.

2.3.6 Wort inoculation: 10 mL of sterile wort was warmed to room temperature and pipetted into sterile flask. One hundred µL (8.2 x 10^8CFU) of inoculated media with *Lactobacillus brevis* culture at absorbance A_{600}=1.0 was added to the wort. On day 3, 7 and 14 inoculated wort was plated on agar plates to verify bacteria were growing.

2.3.7 SIFT-MS analysis: SIFT-MS instrument from Instrument Science, Crewe, UK was used for this study. Precursor ions which this instrument uses are H_3O^+, NO^+ and O_2^+. Samples were analyzed at room temperature [56, 57].

The SIFT-MS has several fluctuating parameters, like temperature, water vapour, pressure, gas flow rate, mass discrimination and diffusion, which must be taken into consideration when measuring samples. Pressure is regulated by a turbo pump and must be kept constant, so the software can properly calculate product ions from reaction coefficients. Flow rate to the inlet is usually constant, but changes could occur when tube length is changed or the flow is modified in some way. Changes in the flow inlet are corrected during the calibration process, because it is of utmost importance to software to obtain accurate counts based on flow rate into the inlet. For larger ions, that are not

counted as accurately as smaller ions, mass discrimination settings are used. For the account of ions that strike the tube wall and are not read by the quadrupole mass filter, diffusion settings are used.

Samples were measured by piercing of aluminum foil cap with needle connected with transfer line to the SIFT-MS. Multi-ion-monitor (MIM) samples were taken for 30 seconds and the full scans (FS) were taken for 5 minutes at *m/z* range 10 to 150.

To identify major product ions MIM scan was used to quantify ethanol and acetic acid production. The ethanol and acetic acid production was measured using H_3O^+ and NO^+ precursors and their hydrates (*m/z* 47, 65 and 83 for ethanol; *m/z* 61, 79 and 97 for acetic acid) were taken into account. FS mode was used to identify other products of importance. All the compounds were identified using SIFT-MS library which is based on numerous studies which investigated reaction products and rate constants of a large range of compounds [58]. The SIFT-MS data was normalized for each experiment as explained by Hryniuk and Ross [59]. For MIM measurements of ethanol and acetic acid, counts for control flask (water only) were subtracted from sample flasks. For the investigation of wort inoculated with *L. brevis*, counts of control flask (wort only) were subtracted from each inoculated sample.

2.3.8 Counting CFU on Petri dishes: On days 3, 7 and 14 after inoculation of ten millilitres wort with one hundred microliters *L. brevis* inoculum, tenfold dilutions were prepared and one hundred microliters of each dilution was plated on Petri dish with MRS agar.

2.4 Results and discussion

2.4.1 Culture headspace spectra of wort using FS mode

Previous studies on malt aldehydes suggested that the compounds in higher concentrations which could cause off-flavour in beer are Stecker‟s aldehydes, linear fatty acid aldehydes and Maillard reaction products [37, 47]. Thus, the headspace of the wort sample was investigated in order to identify the content of aldehydes and possible other volatile compounds. Headspace samples for autoclaved wort were taken and measured in triplicate using FS analysis (Fig. 7 and 8). The compounds of wort were identified through SIFT-MS product ion library, by examination of mass spectrum of all characteristic product ions and cross-referencing with H_3O^+ and NO^+ precursors, when applicable. These included acetaldehydes, 2, 3-butanedione, 2-methylpropanal, and propanol were identified (Table 4). The compound methional, also typical for malt and wort, was not found. However, an additional typical compound for the wort observed was methanethiol. Methional could easily degrade to methanethiol compounds by either decarboxylation reaction or spontaneous degradation [60]. As described in “Methods and materials” section the wort was autoclaved in order to be sterilized for 15 minutes at 121^o C. Therefore, it can be considered that methional degraded to methanethiol.

Pentanal and 2-methylbutanal compounds although typical for wort and malt, in this case are further increased due to inclusion of honey in wort [61, 62].

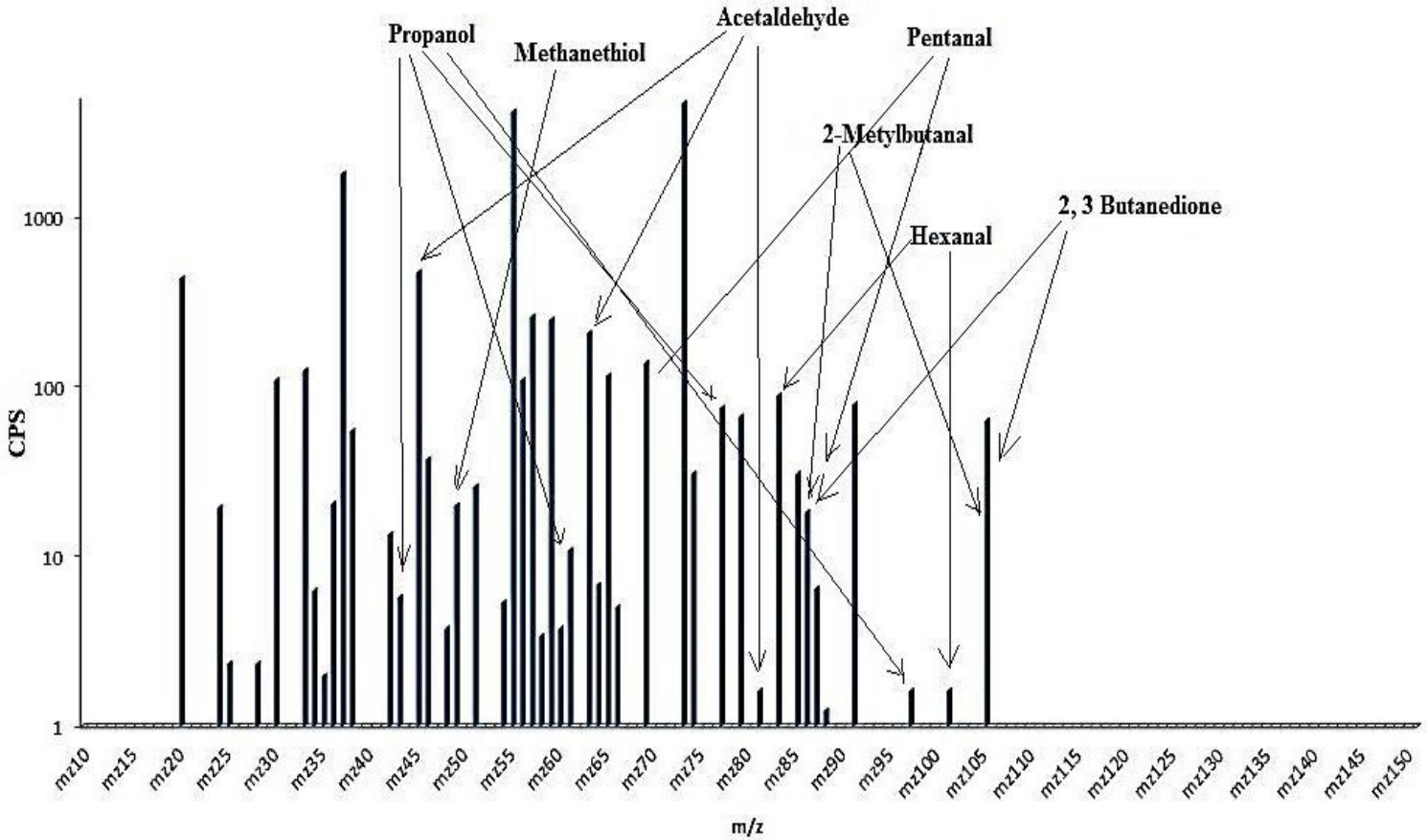

Figure 7. Analysis of gas headspace for wort by SIFT-MS. The spectra were generated using H_3O^+ precursor ions showing the mean of 3 measurements. Ions produced from blank (water) sample were subtracted from this data. Product m/z values for common compounds in wort acetaldehyde, 2,3 – butanedione, hexanal and 2-methylbutanal are indicated on the graph.

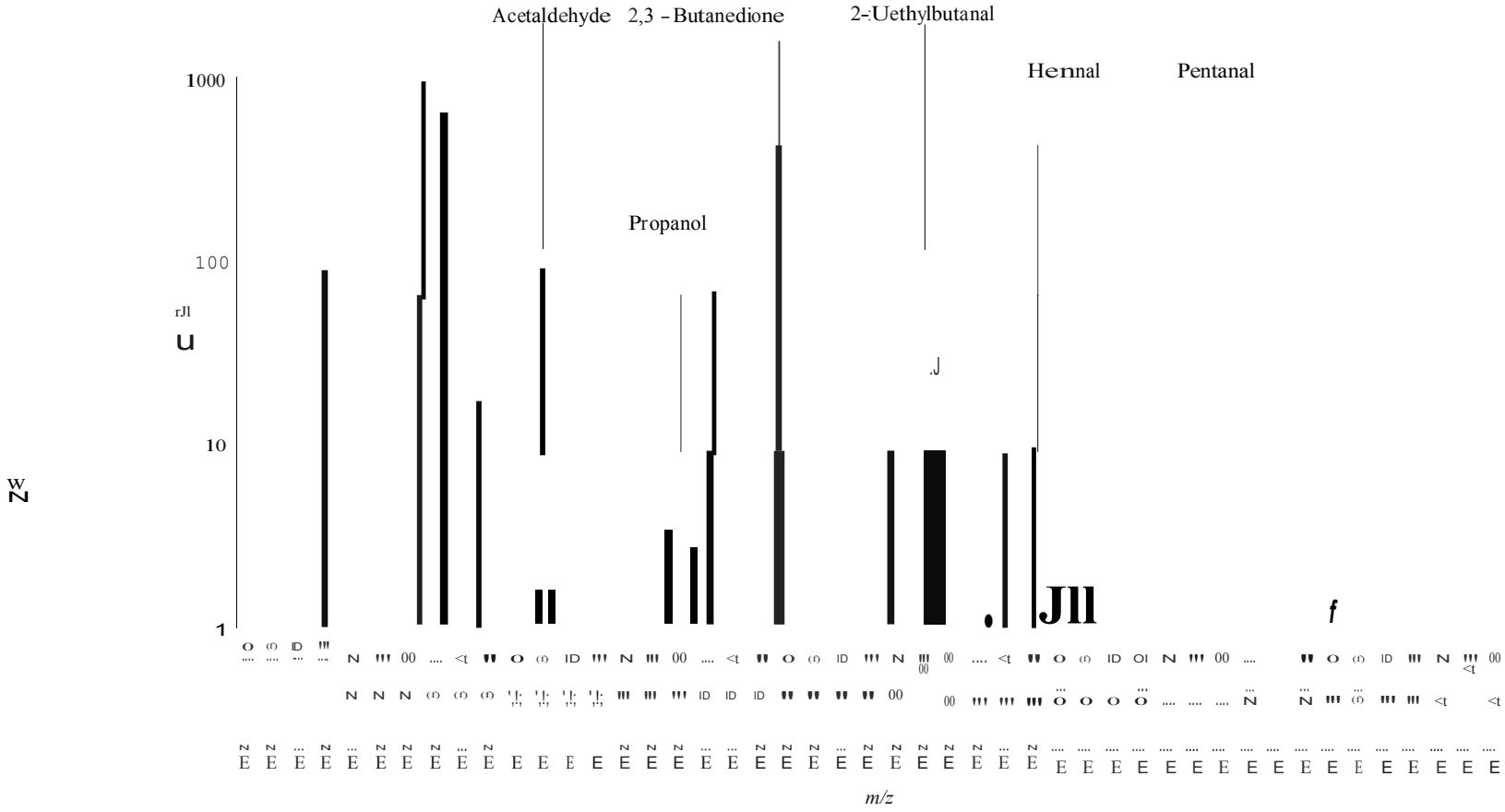

Figure 8. Headspace gas SIFT-MS spectra analysis of wort using NO+ precursor ions. The mean of 3 samples is shown. Ions produced from blank (water) sample were subtracted from this data. Product m/z values for common compounds in wort as conformation of the compounds acetaldehyde, 2,3 – butanedione, hexanal and 2-methylbutanal are indicated on the graph.

Table 4. Summary of putative compounds identified using SIFT-MS in headspace of wort samples. Below are values of H_3O^+ product ion *m/z* with column for hydrates, where applicable. The last column represents NO^+ reaction products for the identified compounds.

Putative compounds	**H_3O^+ Product Ions (*m/z*)**	**H_3O^+ Product Ions Hydrates (*m/z*)**	**NO^+ Product Ions (*m/z*)**	**Reference**
Acetaldehyde	45	63; 81	43	[63]
2- Methylpropanal	73	91	71	[47]
2-Methylbutanal	87	105	85	[47]
Hexanal	83; 101		99	[63]
Propanol	43; 61	79; 97	59	[64]
Methanethiol	49		NR	[65]
2,3- butanedione	87	105	43; 86	[66]
Pentanal	69	87	84	[63]

NR: no reaction

The product ions not included in Table 4 and not indicated on Figures 7 and 8 are H_3O^+ precursor ions having *m/z* 19, 37, 55 and 73 and NO^+ precursor ions having *m/z* 30 and 48.

Further investigation of the volatile compounds originating from wort was done to follow the changes in volatile compounds coming from wort over time at temperature $20^o \pm 2^oC$ (Table 5). On day 3 there were no new compounds present in wort and the abundance of the product ions did not change. On day 7, due to the long exposure of wort at $20^o \pm 2^oC$ some modifications in volatile compounds occur. These changes can be attributed to the high content of sugars in wort and possible spontaneous fermentation in it. Since the flasks with wort samples were not sealed (they had aluminum foil on the top)

it can be that fermentation occurred due to wild yeast. This may explain the decreasing abundance of product ions attributed to acetaldehyde.

Table 5. Identified compounds in wort sample with their abundance during time course of day 3, 7 and 14. The symbols in the table mean: "+++" very abundant (more than 100ppb); "++" moderate abundant (less than 100ppb); "+"less abundant (less than 10 ppb); "-"missing.

Compound	Day 3	Day 7	Day 14
Acetaldehyde	+++	++	+++
2-Methylpropanal	++	+++	++
2-Methylbutanal	++	-	++
Hexanal	+	-	-
Propanol	+	-	-
Methanethiol	++	+	-
2,3 butanedione	++	-	-
Pentanal	++	-	-
Acetic acid	-	++	-
Ethanol	-	+++	+++

During fermentation, part of acetaldehyde is converted to ethanol and acetic acid. Ethanol along with acetic acid is the product of the fermentation process of wild yeast present in the wort. Also, missing product ions for pentanal and 2-methylbutanal can be attributed to the usage of sugars coming from honey. The reduction of 2, 3- butanedione can be attributed probably to the high content of yeast cells and lowered pH from acetic acid [67]. On day 14, acetic acid was already converted to acetaldehyde, likely due to the presence of wild yeast. This may explain the increased abundance of its product ions on the spectrum. Also, the abundance of product ions for ethanol did not change. This

spontaneous fermentation is known also as Lambic fermentation used today in Belgium to produce Lambic sour beer [55].

2.4.2 Culture headspace spectra for wort inoculated with *L. brevis* using FS mode

The headspace samples for autoclaved wort inoculated with *Lactobacillus brevis* were taken and measured in triplicate using FS analysis (Figs. 9, 10 and 11). The compounds of wort inoculated with bacteria were identified using a SIFT-MS product ion library, by examination of mass spectrum of all characteristic product ions and cross-referencing with H_3O^+ and NO^+ precursors, when applicable.

For samples measured on day 3 after inoculation with bacteria (Fig. 9) the most abundant product ions are propanol, ethanol and acetic acid. The positions for these product ions are presented on Table 6. The other product ions with high abundance not indicated on Figure 9 are as a result of precursor ions H_3O^+ possessing *m/z* 19, 37, 55 and 73 and for NO^+ precursor ion *m/z* 30 and 48.

Table 6. Summary of main compounds identified using SIFT-MS in headspace of wort inoculated with *L. brevis* samples along with the m/z of the ions detected which support their presence by reacting headspace gases with H_3O^+ product ion and hydrates. The last column represents NO^+ reaction products as a confirmation for the identified compounds.

Main compounds	H_3O^+ Product Ions (m/z)	H_3O^+ Product Ions Hydrates (m/z)	NO^+ Product Ions (m/z)	Reference
Ethanol	47	65, 83	45	[68]
Propanol	43	61, 79, 97	59	[64]
Acetic acid	61	79, 97	90	[69]

For samples measured on day 7 after inoculation with *L. brevis* (Fig. 10) the abundance of ethanol, propanol and acetic acid product ions is lowered (Table 7). Similarly to the data from the sample measured on day 3 precursor ions are not indicated on the graph.

Table 7. Changes in the quantity of the compounds produced from *L. brevis* in wort through days 3, 7 and 14. Controls (wort) were subtracted from this data.

Day	Ethanol, ppb	Acetic acid, ppb	Propanol, ppb
3	1526	503	65
7	594	27	54
14	340	ND	ND

ND: No detection for this compound on this day after inoculation.

On day 14 after inoculation with bacteria (Fig. 11), the only product ions identified from the spectra are those for ethanol.

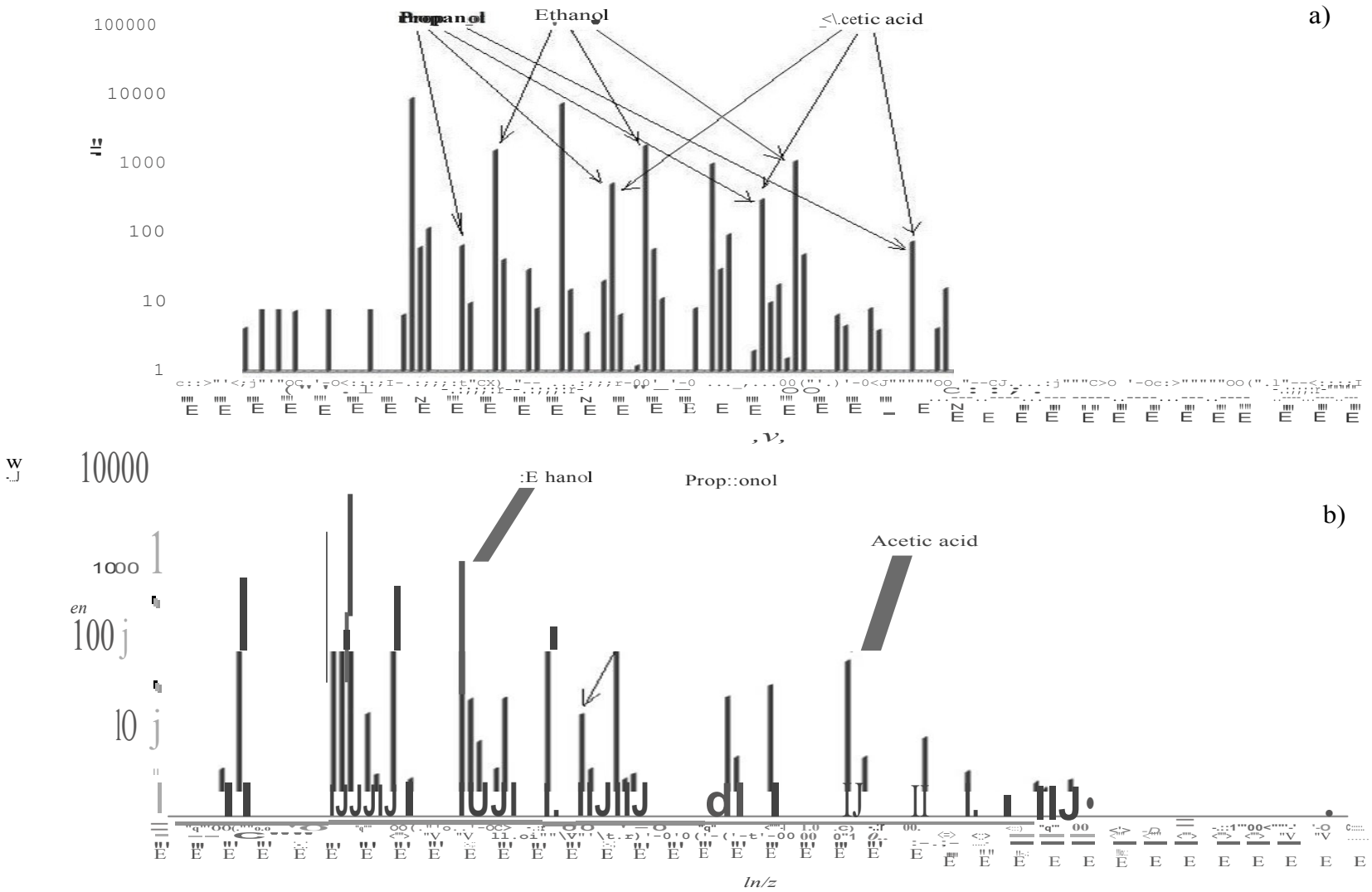

Figure 9. Headspace gas SIFT-MS spectra analysis of wort inoculated with *L. brevis* measured on day 3. Graph a) represents spectra using H_30+ precursor ions and b) represents spectra using NO+ precursor ions. Ions produced from blank (wort) sample were subtracted from this data. Product m/z values for major peaks are indicated on the graph.

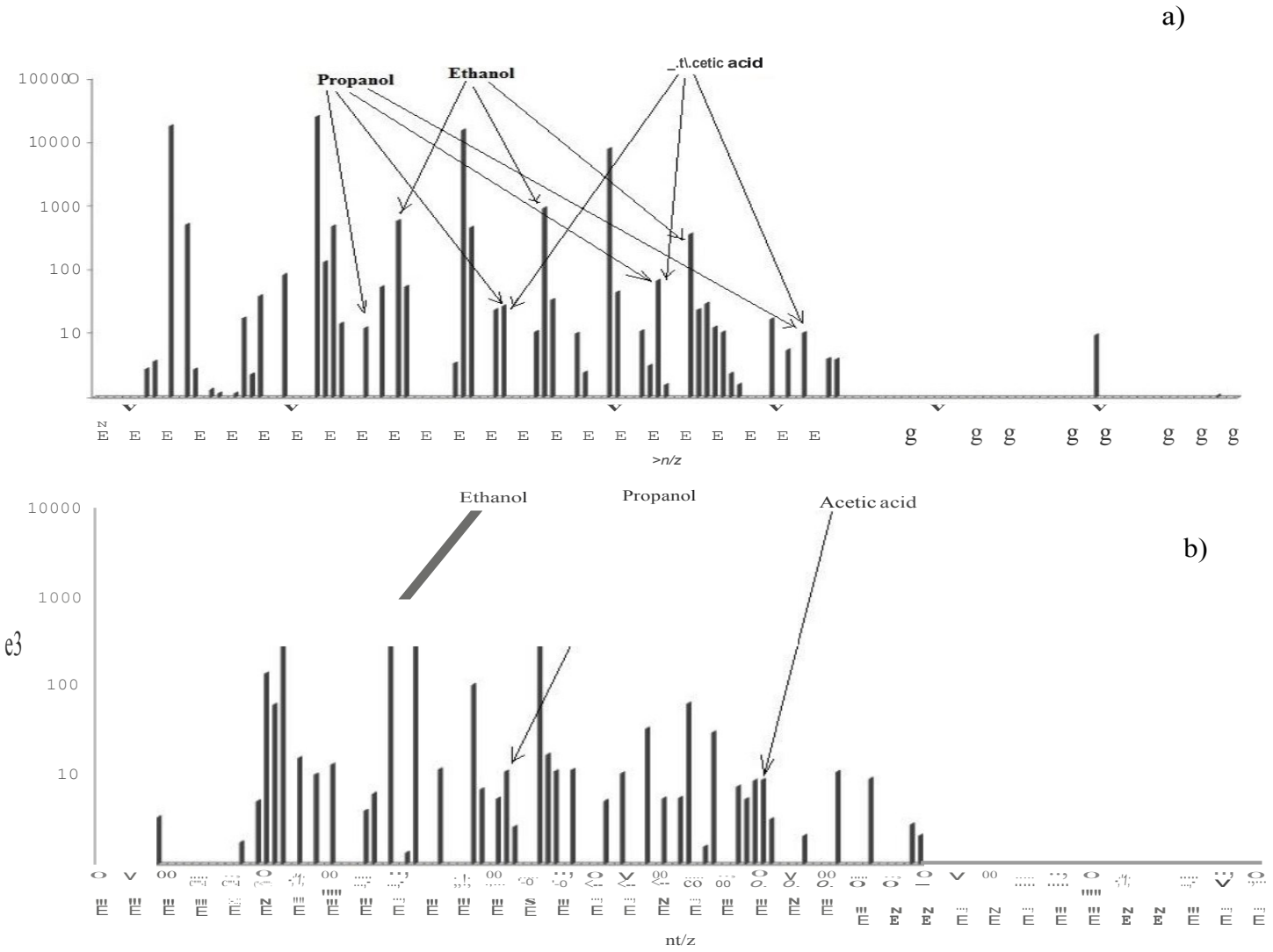

Figure 10. Headspace gas SIFT-MS spectra analysis ofwort inoculated withL. *brevis* measured on day 7. Graph a) represents spectra using H_30+ precursor ions and b) represents spectra using NO+ precursor ions. Ions produced from blank (wort) sample were subtracted from this data. Product *mlz* values for major peaks are indicated on the graph.

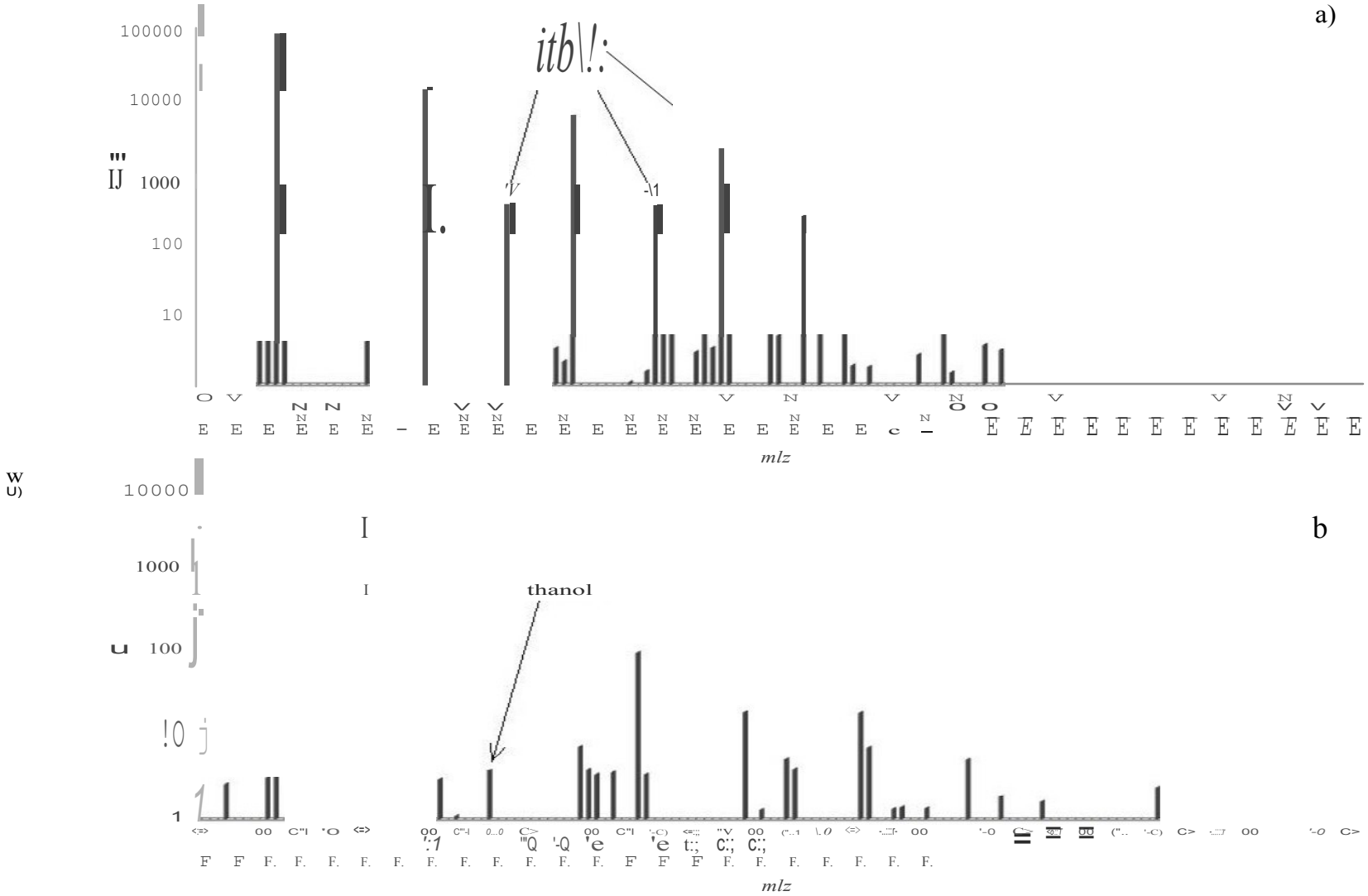

Figure 11. Headspace gas SIFf-MS spectra analysis of wort inoculated with *L. brevis* and measured on day 14. Graph a) represents spectra using H30+ precursor ions and b) represents spectra using NO+ precursor ions. Ions produced :fi:om blank (wort) sample were subtracted :fi:om this data. Product *mlz* values for major peaks are indicated on the graph.

Volatile compounds identified in the headspace of the samples are ethanol, acetic acid and propanol (Fig. 9 and 10). Although, lactic acid was expected to be one of the compounds that resulted from the fermentation of *L. brevis*, lactic acid was not one of the compounds detected in the headspace of the sample. The reason for this is that lactic acid typically does not exhibit enough volatility [70]. According to measurements not presented in this thesis, in order to be detected by SIFT-MS the concentration of lactic acid needs to be at least 1% in water solution.

As can be seen from Table 7 and Fig. 9, on day 3 there is a high production of ethanol and acetic acid. On day 7, the most abundant product ions are those representing ethanol and acetic acid, but their intensity is lower than on day 3. In addition the Petri dishes inoculated with one hundred microliters of the samples showed more CFU on day 3 than on day 7 (Fig. 12). As described in section 2.5.1, when wort is exposed at temperature around $20^{o} \pm 2^{o}C$ it starts spontaneous fermentation due to wild yeast. So, one way to explain this decrease in CFU of bacteria is that on day 7 wild yeasts were already present in the wort sample along with *L. brevis* and they compete with the bacteria. Moreover, as described in section 2.3.1 bacteria were started from frozen stock. Freezing the bacteria probably weakened the inoculum, resulting in population decline during the experiment. In the laboratory strain some mutations may also occur, resulting in poorer survival in wort or beer, than in culture medium.

This explains not only the lower production of ethanol, but also lower CFU of *L. brevis* on same day. Furthermore, on day 14 there were no colonies on the Petri dishes (Fig. 12)

and ethanol content is even lower than day 3 and 7 (Fig. 11). *L. brevis* is used in Lambic fermentation, but it is added to the wort when the yeast cells are dying. Usually *L. brevis* cannot compete with yeast during fermentation, so when wild yeast got into the wort it likely outcompeted the bacteria. Death of bacteria may explain decreased ethanol production.

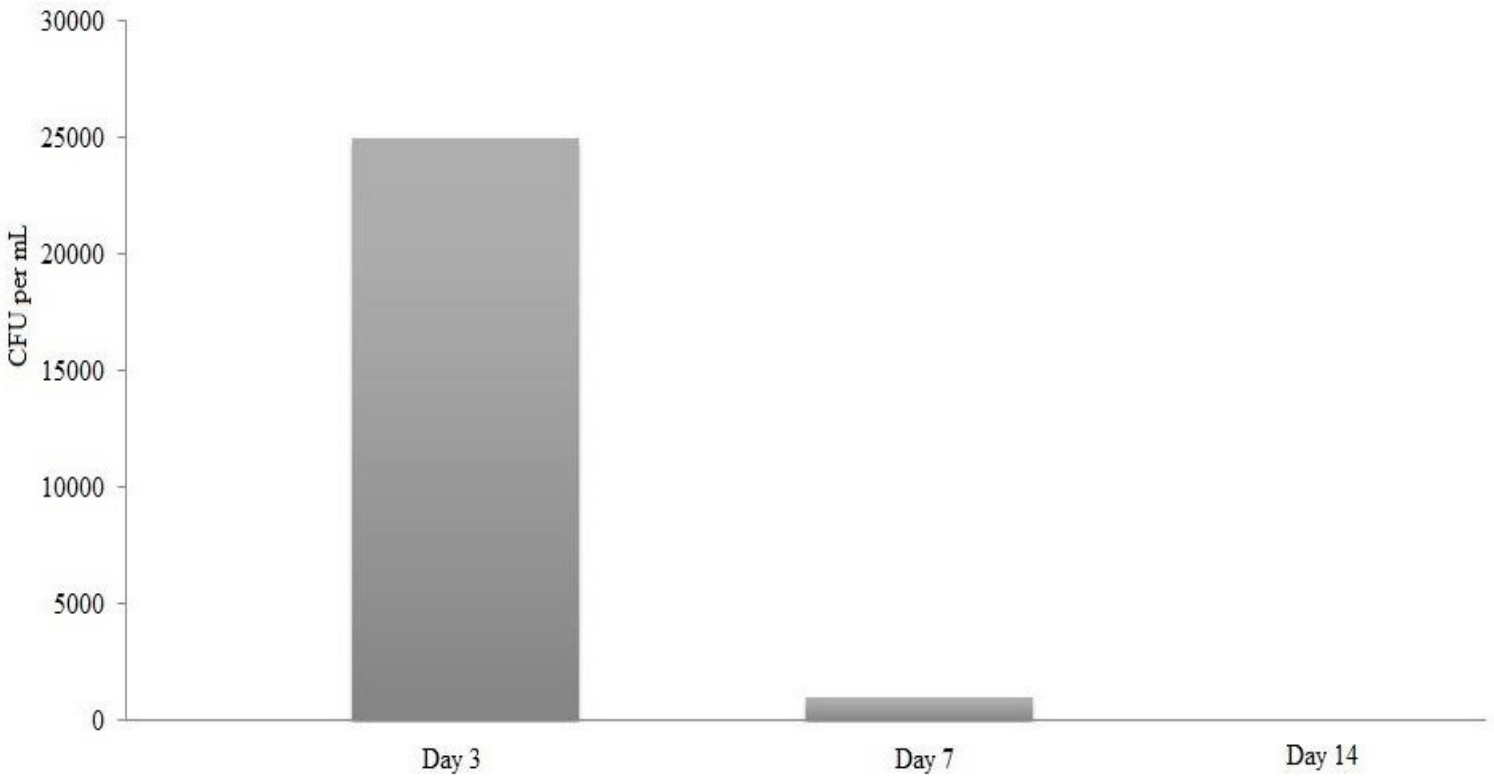

Figure 12. CFU counted on Petri dishes as a result of inoculation with one hundred microliter wort inoculated with *L. brevis* over time course – day 3, 7 and 14.

2.5 Conclusion

The results with wort demonstrated that SIFT-MS can be used for detection of volatile compounds coming from wort and those resulting from fermentation. It was suggested that wort was likely influenced by wild yeast. Moreover, results with wort inoculated with *L. brevis* make it evident that fermentation and ethanol production can be inspected. Results suggest that SIFT-MS can be used for tracking down the production of ethanol during fermentation as well as monitoring the process of fermentation itself. The use of this technique for investigation of wort would be beneficial for breweries and help them with identification and quantification of volatile compounds produced during fermentation.

3. Preliminary investigation of volatile compounds in the headspace of beer inoculated with *Lactobacillus brevis* using SIFT-MS

3.1 Abstract

SIFT-MS was used to identify volatile compounds coming from beer samples and to follow temporal changes in these compounds following inoculation with *Lactobacillus brevis*. The beer degassing prior measurements was necessary due to the high content of ethanol, and carbon dioxide. The already degassed beer was used for inoculation with bacteria. The analysis was done using SIFT-MS with precursors H_3O^+ and NO^+. Full scan mode was used to identify volatile compounds coming from the head space of beer samples and beer inoculated with bacteria. Volatile compounds coming from beer were identified. Product ion possessing *m/z* 39 emerged for samples inoculated with bacteria for 3, 7 and 14 days.

3.2 Introduction

Aroma, along with clarity and taste are one of the most important qualities of beer. But the complexity of beer‟s aroma comes from hops and other ingredients, which are a result of the brewing process. Some of the compounds in small portions give beer the specific fresh taste, however the same compounds in higher concentrations can give off flavours. It is important to identify these compounds present in beer especially during the aging of beer, because they could be related to deterioration. Beer spoilage organisms like lactic acid bacteria, wild yeasts, and some anaerobic bacteria are often present in brewing equipment, in air and raw materials. They can survive years in niches of the brewing equipment (even outside of production stream) without causing spoilage. Then, as a result

of technological fault or insufficient hygiene, these organisms may infiltrate the brewing process and contaminate the entire batch [2]. Most of the bacterial contaminations are due to bacteria that are not pathogenic to humans. There are no established regulations for their control, but their presence in brewing processes can cost a lot of money in breweries all over the world [27]. Bacterial contamination is particularly dangerous for beer, because it develops rapidly and causes spoilage [7]. The most common bacteria responsible for beer spoilage are from the family *Lactobacillus*. Particularly the species *Lactobacillus brevis* is responsible for over 35% of beer spoilage events [5]. Detection of bacterial contamination in early stages is a very important and difficult process [7]. Nowadays, there are several methods used as a way to identify bacterial contamination of beer. Most of them take 1 to 7 days to determine if there is a bacterial contamination [34]. More detailed explanation of these methods can be found in Section 1.1.3.

The SIFT-MS technique works as a chemical ionisation for detection of volatile compounds using three precursor ions (H_3O^+, NO^+ and O_2^+). The ionized products of the reactions of the volatile compounds with precursor ions are detected. SIFT-MS has a great potential for detection of beer spoilage bacteria, because each species likely generates volatile compounds which can be detected in the early stages of the contaminant growth (Table 1) [2]. It is well known fact that *L. brevis* is one of the most common beer spoilage bacteria [5]. The goal of this study is to investigate the effect of *L. brevis* on beer and the use of SIFT-MS to detect volatile metabolites produced by the bacteria in beer headspace.

3.3 Materials and methods:

3.3.1 Bacterial strain: *Lactobacillus brevis* (ATCC 4006) was used in this study as model beer spoilage bacterium. The bacterial culture was started from pellets rehydrated with 1 mL de Man, Rogosa, Sharpe (MRS) Broth. Aseptically rehydrated pellets were transferred into 40 mL liquid MRS Broth. Several drops from this suspension were used to inoculate a second flask of broth. Flasks were incubated at room temperature for 48 hours. After 48 hours the growth was evident by turbidity in the broth. From the inoculated tubes glycerol stock was prepared. Bacterial cultures used for this study were always started from frozen stock.

3.3.2 Glycerol stock for *Lactobacillus brevis*: Eighty percent glycerol with 20% water was autoclaved for 15 min. Then 200 µL of autoclaved 80% glycerol was added to 800 µL of *L. brevis* mixture pregrown in MRS Broth for 24 hours. The mixture was briefly vortexed and stored at -80^{o}C.

3.3.3 Growing the bacteria from frozen stock: The frozen stock was scraped using sterile pipette tip and streaked onto fresh MRS agar plates. The agar plates were incubated for 48 hours at 20^{o}± 2^{o}C. Single colonies were evident in the second and third section of the MRS plate after 48 hours. Using a sterile loop, one colony was picked up and inoculated in 40 mL liquid MRS media. After 48 hours bacteria were growing and turbidity was present in the broth.

3.3.4 Spectrometry: A GE Healthcare Ultrospec 2100 pro UV/Visible Spectrophotometer was used to measure the absorbance of inoculated media at 600 nm.

Fresh medium was used as a blank. For the purpose of this study cultures with optical density (OD) 1.0 was used. Day after inoculation of the media OD was measured. If the OD was higher than needed it was adjusted by adding media to the inoculated sample. Typical sample concentration process of adjustment is explained in section 2.3.4.

3.3.5 Beer: Bottled, ready to drink beer Pale Ale from Sleeping Giant Brewery, Thunder Bay, Ontario with 4.5% alcohol content was used for this study. Beer was degassed for 15 min under vacuum (to remove carbon dioxide and some of the ethanol) and stored at 4^{o}C.

3.3.6 Beer inoculation: Ten mL of degassed beer was pipetted into sterile flask and inoculated with one hundred microliters of *L. brevis* as above. On days 3, 7 and 14 beer (one hundred microliters of inoculated beer) was plated on MRS agar plates and measured with SIFT-MS.

3.3.7 SIFT-MS analysis: SIFT-MS instrument from Instrument Science, Crewe, UK was used for this study. Precursor ions which this instrument uses are H_3O^+, NO^+ and O_2^+. Samples were analyzed at room temperature [68, 69].

Samples were measured by piercing an aluminum foil cap with a needle connected to a transfer line to the SIFT-MS. Multi-ion-monitor (MIM) samples were taken for 30 seconds and the full scans (FS) were taken for 5 minutes at *m/z* range 10 to 150.

To identify major product ions MIM scan was used to quantify ethanol, lactic and acetic acid production. The ethanol, lactic and acetic acid production was measured using H_3O^+ and NO^+ precursors and their hydrates (*m/z* 47, 65 and 83 for ethanol; *m/z* 61, 79

and 97 for acetic acid) were taken into account. FS mode was used to identify other products of importance. All the compounds were identified using SIFT-MS library which is based on numerous studies which investigated reaction products and rate constants of a large range of compounds [58]. The SIFT-MS data was normalized for each experiment as explained by Hryniuk and Ross [59]. For MIM measurements of ethanol and acetic acid, counts for control flask (water only) were subtracted from sample flasks. For the investigation of beer inoculated with *L. brevis*, counts of control flask (beer only) were subtracted from each inoculated sample.

3.3.8. Calculation of relative abundance of product ion possessing m/z 39 in comparison with product ion possessing m/z 37: The relative abundance was calculated following the formula $\frac{\text{relative abundance for product ion with } m/z \text{ 39, \%}}{}$

Where the *m/z* 39 and *m/z* 37 product ions intensity are taken from the raw data of the spectra and are in cps. Product ion having *m/z* 37 represents precursor ion connected with molecule of water having oxygen atom ^{16}O ($H_3O^+.H_2{}^{16}O$) and product ion having *m/z* 39 represents precursor ion connected with molecule of water having oxygen atom ^{18}O ($H_3O^+.H_2{}^{18}O$).

3.4 Results and discussion

3.4.1 Headspace spectra of degassed beer using FS mode

Previous studies show that beer flavour and aroma depends on the complexity of compounds found in beer [71, 72, 73]. The headspace of control degassed beer was analyzed and measured in triplicate using FS analysis (Fig. 13). The compounds detected in beer were identified through SIFT-MS product ion library, by examination of the mass spectrum of all characteristic product ions and cross-referencing with H_3O^+ and NO^+ precursors, when applicable (Table 8). Each of these compounds contributes to the flavour of beer. As mentioned in 3.3.5 beer was degassed to remove some of the ethanol and carbon dioxide and simulate beer going flat. However, this degassing might have removed some other volatile compounds present in beer.

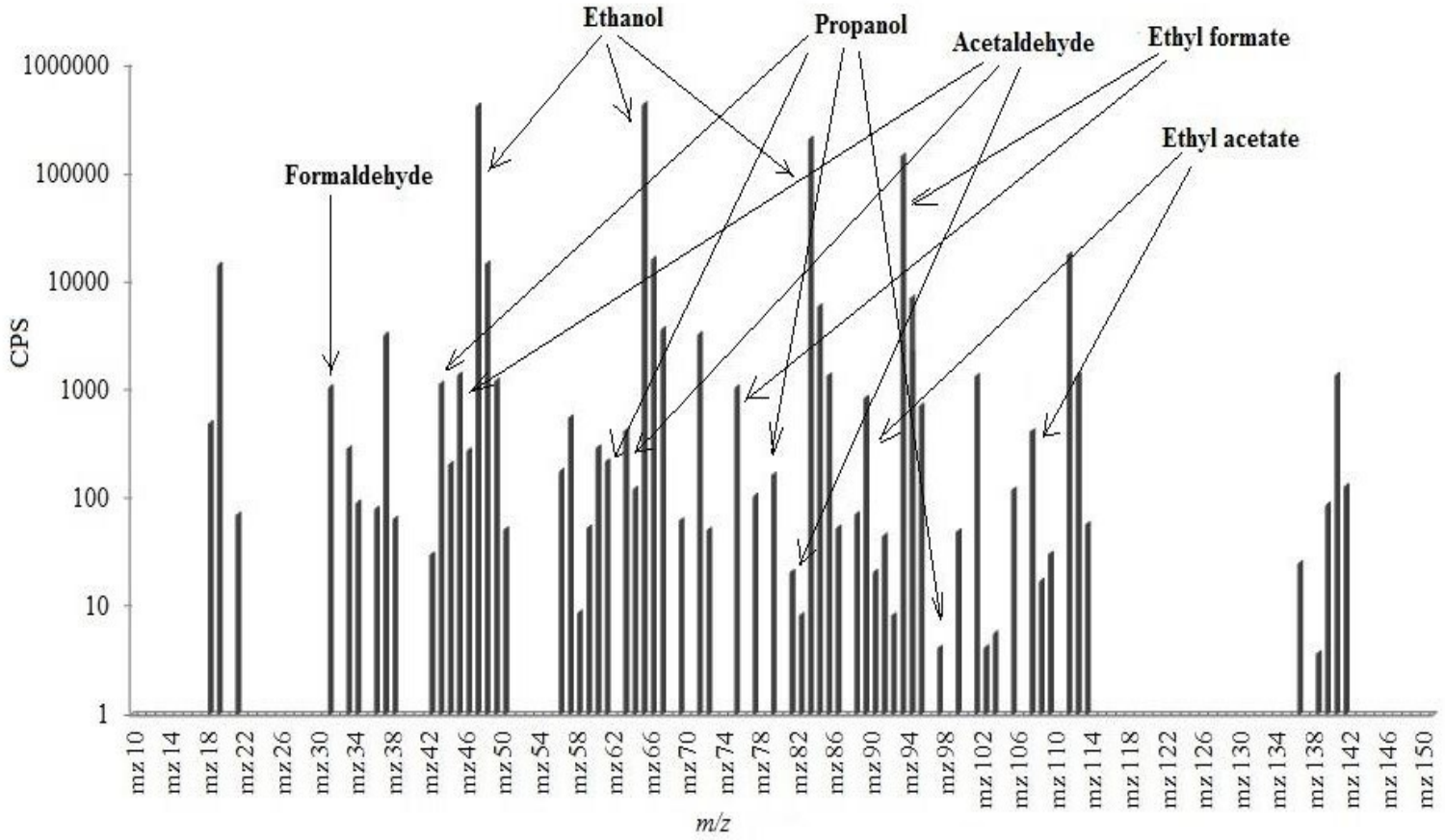

Figure 13. Analysis of gas headspace for beer by SIFT-MS. The spectra were generated using H_3O^+ precursor ions showing the mean of 3 measurements. Ions produced from blank (water) sample were subtracted from this data. Product m/z values for common compounds in beer ethanol, propanol, ethyl acetate are indicated.

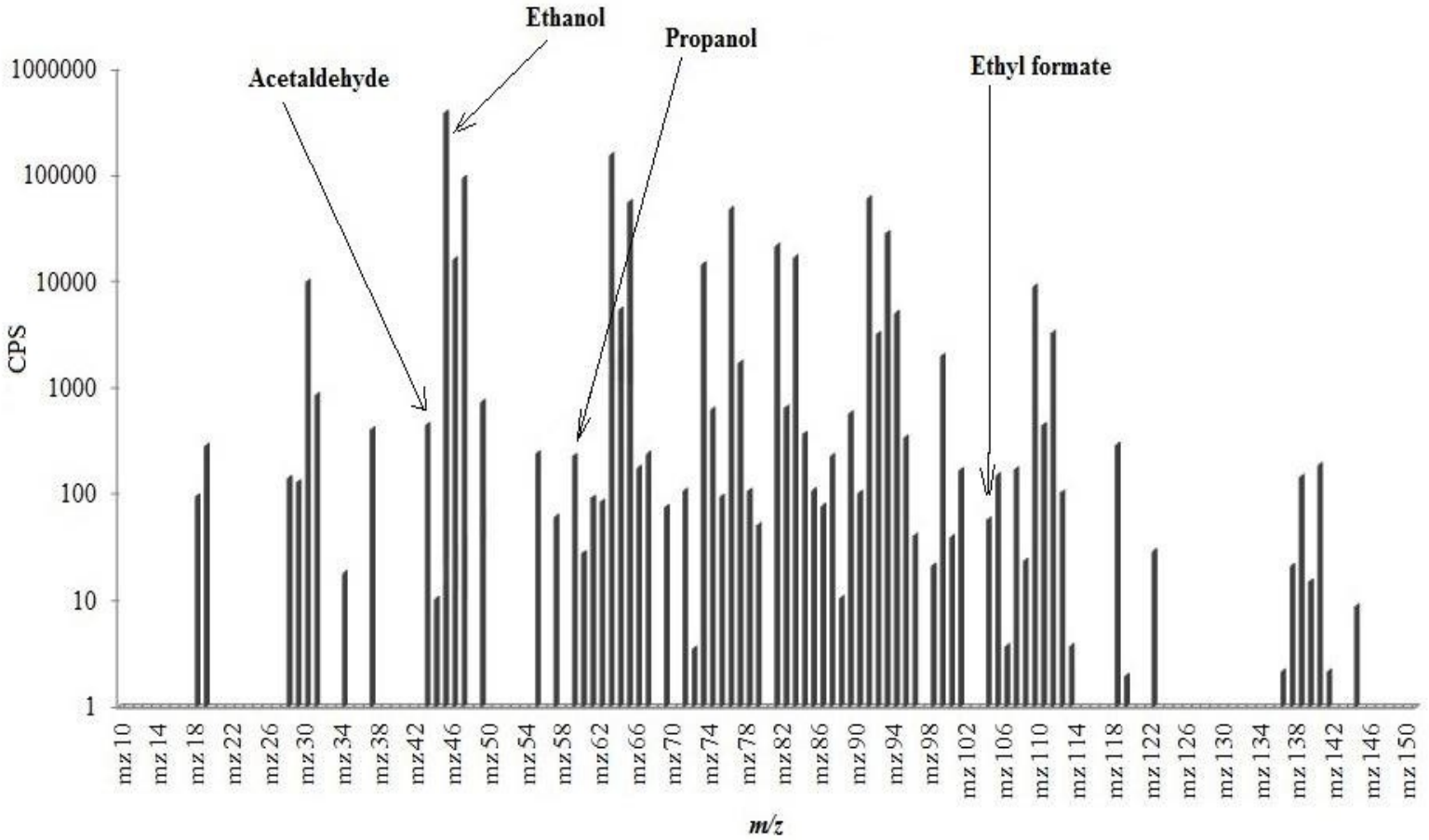

Figure 14. Headspace gas SIFT-MS spectra analysis of beer using NO^+ precursor ions. The mean measurements of 3 samples is shown. Ions produced from blank (water) sample were subtracted from this data. Product m/z values for common compounds in beer as conformation of the compounds ethanol, propanol and ethyl acetate are indicated.

Table 8. Summary of putative compounds identified using SIFT-MS in headspace of beer samples along with the *m/z* of the ions detected which support their presence by reacting headspace gases with H_3O^+ product ion and hydrates. The last column represents NO^+ reaction products for the identified compounds.

Putative compounds	**H_3O^+ Product Ions (m/z)**	**H_3O^+ Product Ions Hydrates (m/z)**	**NO^+ Product Ions (m/z)**	**Reference**
Formaldehyde	31		NR	[63]
Propanol	43, 61	79, 97	59	[64]
Acetaldehyde	45	63, 81	43	[63]
Ethanol	47	65, 83	45	[68]
Methanethiol	49	67, 85	NR	[65]
2-Methyl-1-propanol	57	75	73	[81]
Acetone	59	77	88	[63]
Acetic acid	61	79, 97	90	[69]
3-Methyl-1 butanol	71	89	71, 87	[85]
Ethyl formate	75		104	[69]
Ethyl acetate	89	107	43, 130	[75]
Valeric acid	85	103	71, 113	[69]
Phenol	95	113	94, 112	[81]

NR: no reaction for some compounds when using NO^+.

Ethanol together with other fusel alcohols contributes to the alcoholic warm feeling of drinking beer [71]. Ethanol plays important role in the perception of other compounds in beer and overall taste of beer [74]. The high ethanol concentration in beer samples increases odour threshold of the compounds with and make them easier to be detected with SIFT-MS.

Shown on Fig. 13 is the difference between the precursor ions present in water and the same present in beer, as the ambient (water) was subtracted from the spectra and

data was normalized. The explanation of the spectra starts with the three body association (association of MH^+ ions with H_2O molecules) reaction of H_3O^+ ions in water with ethanol [75]. Usually the reaction of ethanol with H_3O^+ precursor is as follows:

$$H_3O^+ + C_2H_5OH \longrightarrow C_2H_5OH_2^+ + H_2O \qquad (1)$$

Following reaction (1) the ethanol product ion having *m/z* 47 is formed. In the presence of water, due to three-body association protonated ethanol undergoes a number of association reactions with water and forms monohydrate (*m/z* 65) and dihydrate (*m/z* 83) [75]. Three body association reactions can be described as follows:

$$H_3O^+ + H_2O \longrightarrow H_3O^+.H_2O \qquad (2)\ [75]$$

Produced hydrated ions ($H_3O^+.H_2O$) can act as precursor ions, when they react, with trace gas molecule M and form product ions like $MH^+.\ H_2O$. Sometimes they can even form $MH^+.\ (H_2O^+)_{2,3}$ product ions. Ethanol does not react with hydrated ions $H_3O^+.\ (H_2O)_3$. Usually, in high ethanol presence precursor depletion should be more than 90%. However, the calculated precursor depletion for sample with degassed beer is 43.5%. Calculation was done as follow:

<u>For water:</u>

m/z 19 = 310 083.8 cps

m/z 37 = 121 591.6 cps

m/z 55 = 49 510.2 cps

m/z 73 = 13 660.1 cps

X_{water} = *m/z* 19 + *m/z* 37 + *m/z* 55 + *m/z* 73 = 494 846.1 cps

For beer:

m/z 19 = 137 940.5 cps

m/z 37 = 53577.5 cps

m/z 55 = 19748.5 cps

m/z 73 = 3962.1 cps

Y_{beer} = *m/z* 19 + *m/z* 37 + *m/z* 55 + *m/z* 73 = 215 228.5 cps

Depletion = Y_{beer}/X_{water} *100% = 215 228.5/494 846.1 * 100% = 43.5 %

The difference between expected precursor depletion and the calculated one may be due to degassing of beer (some of the ethanol was removed along with other volatile compounds present in beer). Although, the ethanol content was lowered due to degassing of beer, it was still high enough to act as a precursor forming ions $C_2H_5OH_2^+$ $(C_2H_5OH)_{1,2,3}$ that reacted the same way as hydrated ion $H_3O^+.H_2O$ [76]. Formation of $C_2H_5OH_2^+$ $(C_2H_5OH)_{1,2,3}$ ions explains the increased abundance on ions having *m/z* 93, *m/z* 111and *m/z* 139 (Fig. 13) as they are the result of high ethanol content [83]. These product ions with high abundance are a result of the formation of clusters of other compounds detected in beer with $C_2H_5OH_2^+$ $(C_2H_5OH)_{1,2,3}$ ions. Product ion having *m/z* 93 is $(C_2H_5OH)_2H^+$ and product ion with *m/z* 111 is its monohydrate $(C_2H_5OH)_2H^+H_2O$. Product ion having

m/z 139 is protonated ethanol clustered with three ethanol molecules $C_2H_5OH_2^+(C_2H_5OH)_3$ [83]. This product ion in high ethanol concentration is dominant. However, on the spectra for degassed beer it is present but not dominant. This is an evidence that some of the ethanol was removed during the degassing of beer, resulting not only in lowered product ion having *m/z* 139, but also higher abundance of ethanol monohydrate (*m/z* 65) and lower abundance of ethanol dihydrate (*m/z* 83).

The low quantity of formaldehyde was detected due to malt treatment [7]. In order to speed up the process of malt preparation, grains were treated with formaldehyde. This is a common technique used by factories producing malt and the reason for having trace of formaldehyde in final beer. Formaldehyde is a typical component of beer present in very low quantities [77]. Due to the high ethanol content in beer, formaldehyde reacts with ethanol molecule forming CH_2O^+ (C_2H_5OH) product ion having m/z 77.

$$CH_2O + C_2H_5OH_2^+ (C_2H_5OH)_2 \longrightarrow CH_2O^+(C_2H_5OH) + 2\ C_2H_5OH$$

Similar to formaldehyde, acetone is found in beer in small concentrations. Typical concentrations of acetone in beer are 0.0174 – 1.7 mg/L [78]. Detected acetone in the sample may be due to the presence of ethyl acetate as described in some studies [74]. Ethyl acetate contributes to the rich flavour of beer and the acceptable levels for it in beer are 10 – 60 mg/L [74]. From Fig. 13 can be seen product ions for ethyl acetate. Ethyl acetate molecule reacted with hydrated ions and produced protonated ethyl acetate (reaction (3)).

$$H_3O^+.(H_2O)_{1,2,3} + CH_3COOC_2H_5 = CH_3COOC_2H_5H^+.H_2O + 1, 2, 3\ H_2O \quad (3) \qquad [75]$$

Detected product ions for ethyl acetate are presented in Table 8. $C_2H_5OH_2^+(C_2H_5OH)_2$ did not react with ethyl acetate to form cluster ions, but it might be considered that ethyl acetate present in the sample is due to the reaction between ethanol and acetic acid.

Acetic acid detected in the samples is likely a result of the fatty acid metabolism of yeast during fermentation. Along with ethanol, acetic acid is an important component in the formation of the flavour-giving ethyl acetate [78].

Propanol is a common compound in beer. Fig.13 shows that propanol was detected. Propanol along with high esters contributes for the "alcoholic" flavour of beer [71, 73]. It concentration varies from 8 – 33 mg/L in different types of beer [78]. In comparison with ethanol molecules, propanol molecules can react with hydrated ions and produce monohydrate and dihydrate (Table 8).

High temperature during the fermentation process of beer production increases the formation of acetaldehyde, but also increases the rate of its degradation. That is why in final beer, the content of acetaldehyde is significantly lower than in wort and during fermentation. The overall content of this component in beer depends on the pitching rate of the yeast (amount of yeast added to wort; the rate is measured in millions yeast cells per millilitre of wort) [72]. Higher pitching rate decreases the concentration of acetaldehyde in beer [72].

Hops contain essential oils which includes turpentine oil and stereoptene valerol. The first compound is very volatile and disappears entirely during the brewing process; whether the second compound gives a mixture of stereoptene termed valerol and resin and easily

oxidizes in air forming valeric acid. The presence of valeric acid confirms that hops were used in the process of beer brewing, but not some artificial additives to achieve the bitter flavour [79, 80].

3.4.2 Culture headspace spectra for beer inoculated with *L. brevis* using FS mode

Some of the compounds found in beer inoculated with *L. brevis* differ from compounds found in a sample with beer only (Table 9). Also, the changes of these products are presented in Table 10. The abundance of listed compounds was followed on days 3, 7 and 14 after inoculation of the sample with bacteria.

Table 9. Summary of main compounds identified using SIFT-MS in headspace of beer samples inoculated with *L. brevis* during the investigated period day 3, 7 and 14. Below are values of H_3O^+ product ion *m/z* with column for hydrates, where is applicable. The last column represents NO^+ reaction products for the identified compounds.

Main compounds	H_3O^+ Product Ions (m/z)	H_3O^+ Product Ions Hydrates (m/z)	NO^+ Product Ions (m/z)	Reference
Formaldehyde	31		NR	[63]
Acetaldehyde	45	63, 81	43	[63]
Ethanol	47	65, 83	45	[68]
Lactic acid	45, 91		118, 120	[69]
Acetic acid	61	79, 97	90	[69]
Phenol	95	113	94, 112	[81]
Ammonia	18	36, 54	NR	[76]
Ethyl formate	75	94	104	[75]
Methanethiol	49	67, 85	NR	[65]

NR: no reaction for some compounds when using NO^+.

On Figures 15, 16 and 17 are presented the FS spectrum for degassed beer samples inoculated with *L. brevis*.

On day 3 the spectra (Fig. 15a and 16a) are populated with these compounds: ethanol, acetaldehydes, lactic acid, ethyl formate, ammonia, methanethiol and phenol. Other product ions with high abundance are a result of high ethanol content in the sample. Similarly to the control sample degassed beer product ions having *m/z* 93, 111 and 139 are related to high ethanol content and $C_2H_5OH_2^+$ $(C_2H_5OH)_{1,2,3}$ ions (Figs. 15a and 16a). Ethanol and lactic acid are products of the fermentative pathway of bacteria. In contrast to wort contaminated with *L. brevis* (Fig.9, 10 and 11), the samples (day 3 and 7) of contaminated beer produced detectable levels of lactic acid vapour, suggesting higher than 1% concentration in the beer solution (Figs. 15, 16 and Table 10). Ammonia product ions (*m/z* 18, 36 and 54) are due to arginine found in beer and reaction between it and bacteria [82]. Formaldehyde, methanethiol, and phenol as discussed previously are compounds found in final beer in small quantities.

Due to the fermentative pathway of the bacterium some of the compounds were converted. On day 7 (Fig. 15b and Fig. 16b) the product ions of ethanol, methanethiol, ammonia, and phenol are no longer present in the sample. Instead, the spectra showed an increased abundance of acetaldehyde, lactic acid and acetic acid (Table 10). This suggests that ethanol, together with other compounds, was converted to the products detected in Fig. 15b. In addition, in the absence of ethanol the abundance for product ions characterizing precursors H_3O^+ and NO^+ increased (Figs. 15 and 16).

On day 14 (Fig. 17) only acetic acid product ions were detected and they were very abundant (Table 10). The product ions related to the precursors H_3O^+ (*m/z* 19, 37, 48, 55 and 73) and NO^+ (*m/z* 30 and 48) were all present (Fig. 17).

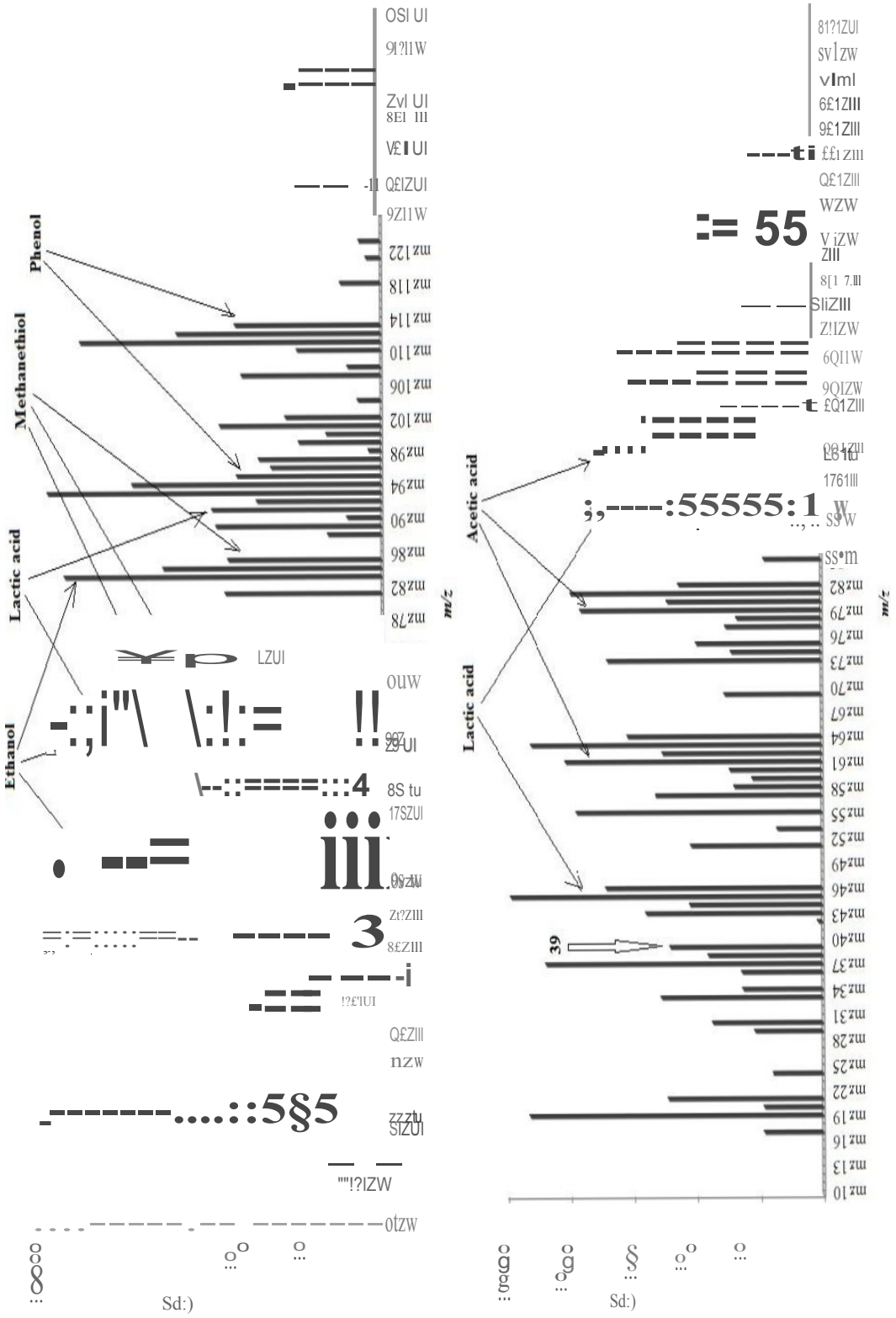

Figure 15. Analysis of gas headspace for beer inoculated with *L. brevis* using SIFT-MS H_3O^+ precursor ion. The spectra on a) represents day 3 and spectra on b) represents product ions produced on day 7 after inoculation. Ions produced from blank (beer) and ambient (water) were subtracted from this data.

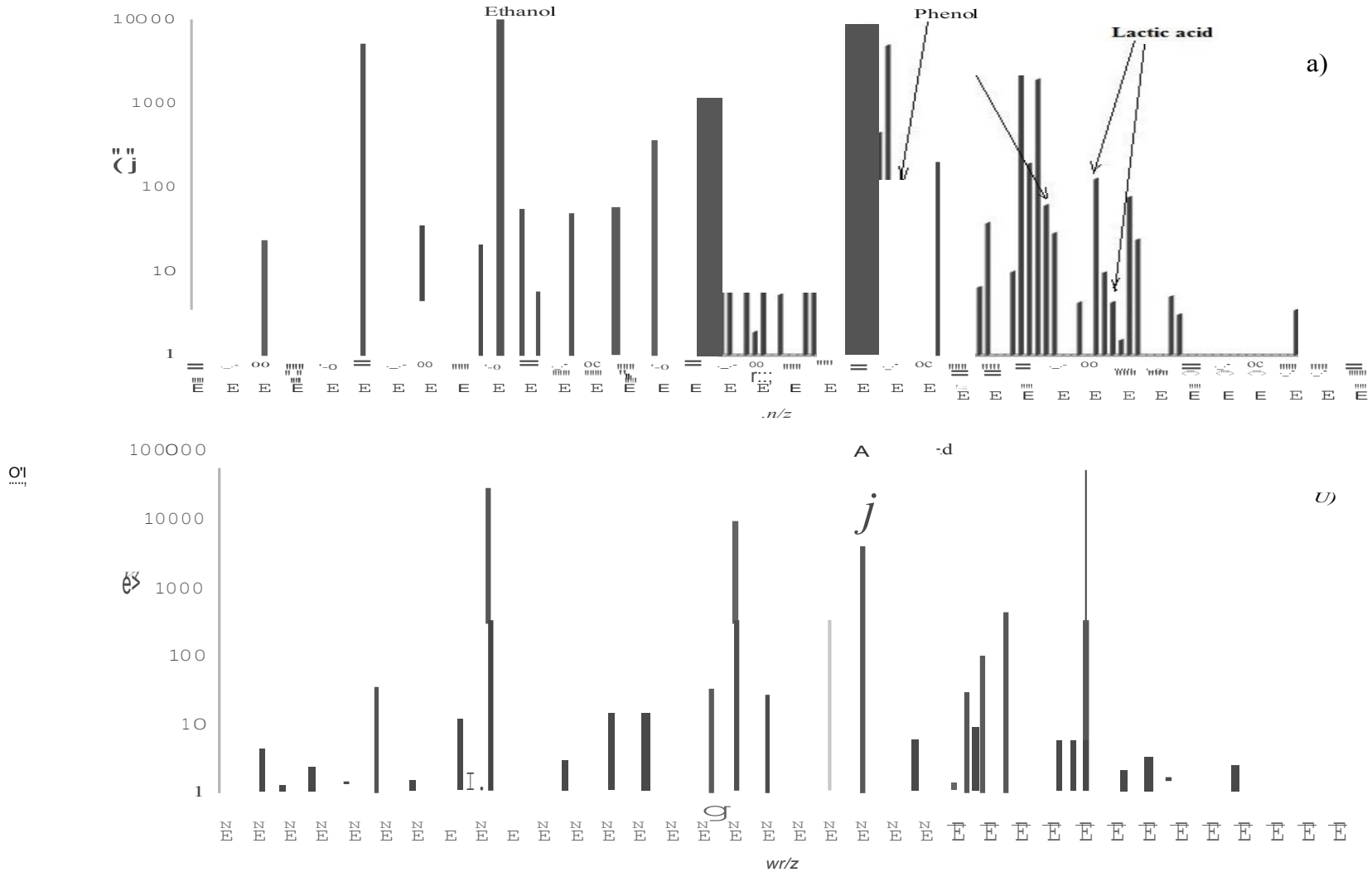

Figure 16. Analysis of gas headspace for beer inoculated with *L. brevis* using SIFT-MS NO^+ precursor ion. The spectrum on a) represents day 3 and spectrum on b) represents product ions produced on day 7 after inoculation. Product ions produced from control (beer) and ambient (water) were subtracted from this data.

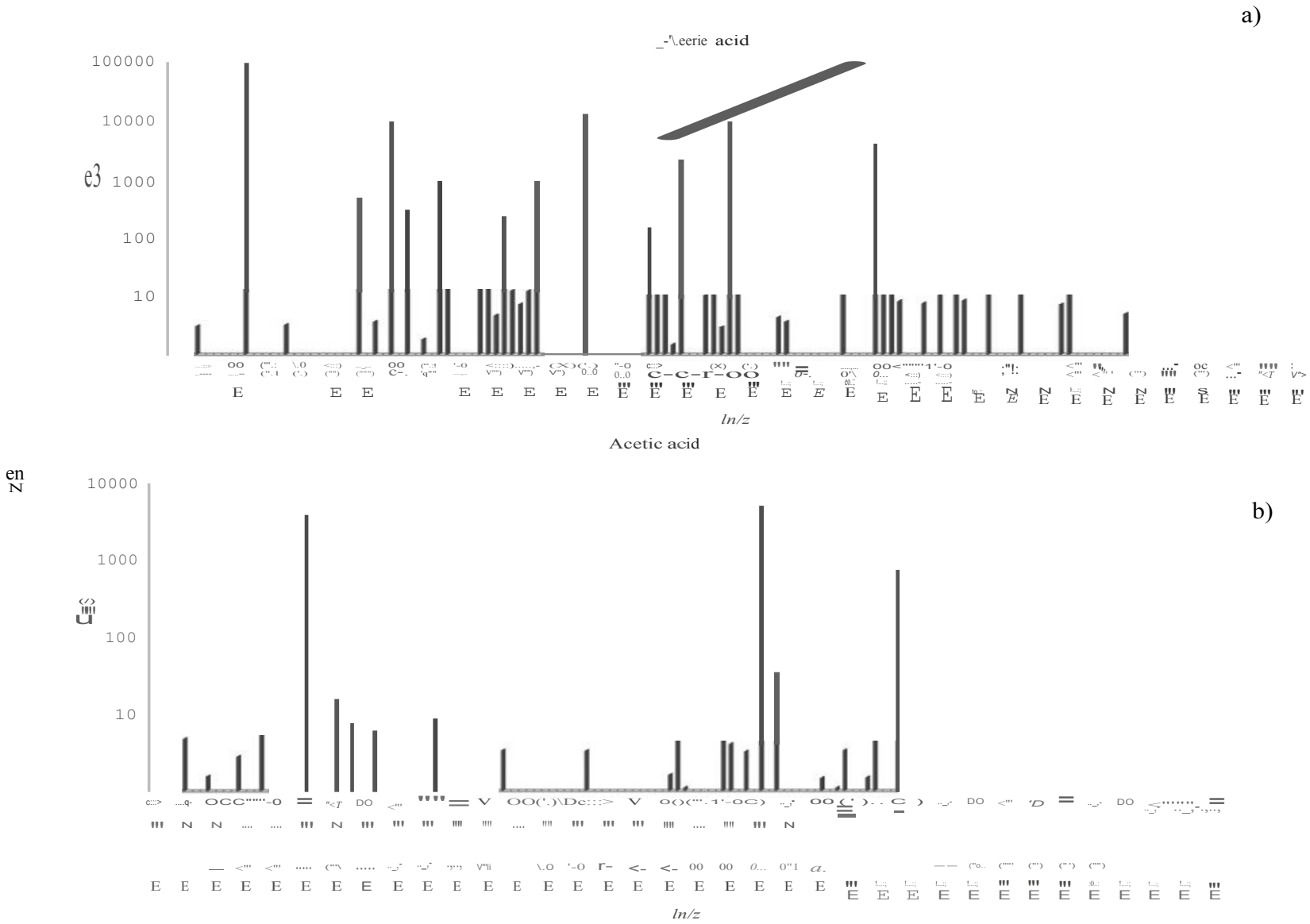

Figure 17. Analysis of gas headspace for beer inoculated with *L. brevis* using SIFT-MS on day 14 after inoculation. The spectra on a) day 14 analysed with H_30+ precursor ions and spectra on b) represents 14 days after inoculation using NO+ precursor ions. Control (beer) and ambient (water) were subtracted from these data.

Table 10. Summary of product ions detected using FS mode on SIFT-MS in beer sample inoculated with *L. brevis* in the period day 3, 7 and 14 and their abundance in this time. "+++" means very abundant (over 10 ppm); "++" means moderate abundance (less than 10ppm); "+" means less abundant (less than 1ppm); "-"means product ion is missing.

Compound	Day 3	Day 7	Day 14
Ethanol	+++	-	-
Lactic acid	+	+++	-
Acetic acid	-	++	+++
Ethyl formate	++	+	-
Acetaldehyde	+	++	-
Ammonia	+	-	-
Methanethiol	+	-	-
Phenol	+	-	-

Along with the product ions identified from the samples with degassed beer inoculated with *L. brevis*, there was another product ion possessing *m/z* 39 that can be seen on the spectrum. This product ion was not present on the control beer sample with subtracted ambient. However, it appeared on the spectrum with normalized data without subtracted ambient and in the raw data as well. Table 11 presents the values (in cps) of this product ion having *m/z* 39 from the raw data of beer samples, where calculations were done for beer sample for days 1, 3, 7 and 14.

Table 11. Calculated relative abundance of product ion possessing *m/z* 39 in comparison with product ion *m/z* 37 for beer samples over time – day 1, 3, 7 and 14. Samples were on temperature 21°C± 2°C. The data in the middle columns is mean raw data from 12 samples without subtracted ambient (water).

Sample	Intensity *m/z* 37, cps	Intensity *m/z* 39, cps	Relative abundance of *m/z* 39, %
Beer day 1	36021.4	143.7	0.397
Beer day 3	46954.3	191.29	0.405
Beer day 7	88102.9	358.6	0.405
Beer day14	110368	523.2	0.472

Product ion having *m/z* 37 represents precursor ion H_3O^+ connected with water molecule having oxygen atom ^{16}O ($H_3O+.H_2{}^{16}O$). Product ion having *m/z* 39 likely represents precursor ion H_3O^+ connected with water molecule having oxygen atom ^{18}O ($H_3O+.H_2{}^{18}O$). In nature the relative abundance of ^{18}O is about 0.2%. However, in mass spectra this relative abundance is 0.4%. According to Table 11, the relative abundance of *m/z* 39 to *m/z* 37 is around 0.4% for day1, 3 and 7. It can be concluded from there that some of this product ion is due to the presence of hydrate ion ($H_3O^+.H_2\ ^{18}O$). The precursor ion H_3O^+ reacted with a water molecule in which the oxygen atom is ^{18}O instead of ^{16}O. This resulted in product ion possessing *m/z* 39. There is an increase in relative abundance on day 14, but this change may be contributed to the aging of beer or oxidizing of sample over time.

The same product ion having *m/z* 39 appears again in the samples with degassed beer inoculated with *L. brevis*. Calculation of the relative abundance of this product ion can be seen from Table 12.

Table 12 Calculated relative abundance of product ion *m/z* 39 in comparison with *m/z* 37 for beer sample inoculated with *L. brevis* for day 3, 7 and 14 of inoculation. Two middle columns are mean values from 12 samples for each day of inoculation. Presented data is raw without subtraction of ambient (water) and blank (beer) samples.

Sample	Intensity *m/z* 37 cps	Intensity *m/z* 39 cps	Relative abundance of *m/z* 39, %
Beer inoculated with *L. brevis* on day 3	57849. 9	281.6	0.486
Beer inoculated with *L. brevis* on day 7	93128.03	457.12	0.491
Beer inoculated with *L. brevis* on day 14	114826	617.4	0.538

The calculated relative abundance in the sample is over the limit of 0.4 %. This means that this product ion cannot be attributed to the hydrated ion ($H_3O^+.H_2\ ^{18}O$), but can be considered as a result of the bacterial contamination in beer. I.e. some of this peak may be due to an unidentified volatile component.

The comparison between the results for investigated samples with beer only and beer inoculated with bacteria revealed that even increased, the relative abundance of product ion possessing *m/z* 39 for beer sample (day 14) is much lower than the relative

abundance of the same product ion for beer sample inoculated with *L. brevis* on day 14. Although, the chemical structure of this compound was not confirmed, product ion having *m/z* 39 may be a result of a compound present in the final beer due to contamination with *L. brevis*. Another explanation is that product ion possessing *m/z* 39 might be a fraction of bigger molecule. The scan was performed from *m/z* 10 to *m/z* 150, so as an improvement for of this experiment, scanning a wider *m/z* range may be suggested. Additional experiments are needed to identify the chemical structure of this putative compound.

3.5 Conclusion

The results for beer samples show that ethanol reacted quickly with H_3O^+ precursor ions and as a consequence created hydrated ions, which further reacted with the rest of the beer components. It is well known fact [83] that the presence of ethanol in a sample investigated with SIFT-MS technique facilitates the detection of these components.

Beer samples inoculated with *L. brevis* revealed volatile compounds typical for fermentative pathway of this bacterium [84]. Moreover, SIFT-MS method was able to detect each volatile compound produced during the course of this experiment. In addition, there was unidentified product ion having *m/z* 39 that appeared on the spectrum. Further calculation and investigation of this product ion are needed to determine its chemical structure, but so far it might be considered as a result of compound formed due to presence of *L. brevis* in beer.

The results show that SIFT-MS can successfully be used for the identification of volatile compounds found in final beer and beer contaminated with bacteria. All volatile compounds coming from spoilage organisms are possible to be detected.

4. Conclusion and future work

Selected Ion Flow Mass Spectrometry was shown to work as a detection method for volatile compounds coming from the headspace of samples with wort and beer. Both sets of experiments gave interesting results which can be considered as a foundation for future research.

Experiments with wort showed that when using this technique it is possible to identify aldehydes and other compounds emanating from wort. Moreover, investigated samples with wort inoculated with bacteria showed the potential of this method for detection of bacterial metabolites. Further investigation with other bacteria and in different quantities is needed for this method to be improved. Further investigation with other bacteria will give the possibility of creating a database with volatile compounds expected from beer spoilage bacteria. Also, SIFT-MS is able to detect volatile compounds that come from wort‟s ingredients which feature is of paramount importance when new additives are planned to be added. This method can be beneficial not only as a detection method of bacterial contaminants, but also as screening method for the compounds in wort. In addition to the previously mentioned benefits, SIFT-MS can also successfully be used as a method for monitoring fermentation of wort. This technique would be useful for breweries for improving not only hygiene but also technological processes.

Results from investigated samples with beer and beer inoculated with bacteria showed that SIFT-MS can be used for detection of volatile compounds from headspace of beer sample and also, for the investigation of beer spoilage bacteria. Presence of the

product ion possessing *m/z* 39 (when using H_3O^+ precursor ions) encourages because it might be considered as a part of compound produced due to beer spoilage bacterium *Lactobacillus brevis*. As an improvement for this experiment I suggest using different quantity of bacteria and follow the same protocol and using an additional technique to identify the chemical structure of this compound. Also, different bacteria can be investigated and create a database with the detected volatile compounds related to different bacteria. Again, this database will be beneficial for breweries for investigation of possible contamination in their products. Furthermore, SIFT-MS can be used for investigation of aldehydes during aging of the beer, which will help breweries to keep high quality of their products.

This Master‟s project was preliminary experiment and demonstrates that SIFT-MS can be used for detection of volatile compounds coming from wort and beer contaminated with bacteria.

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