

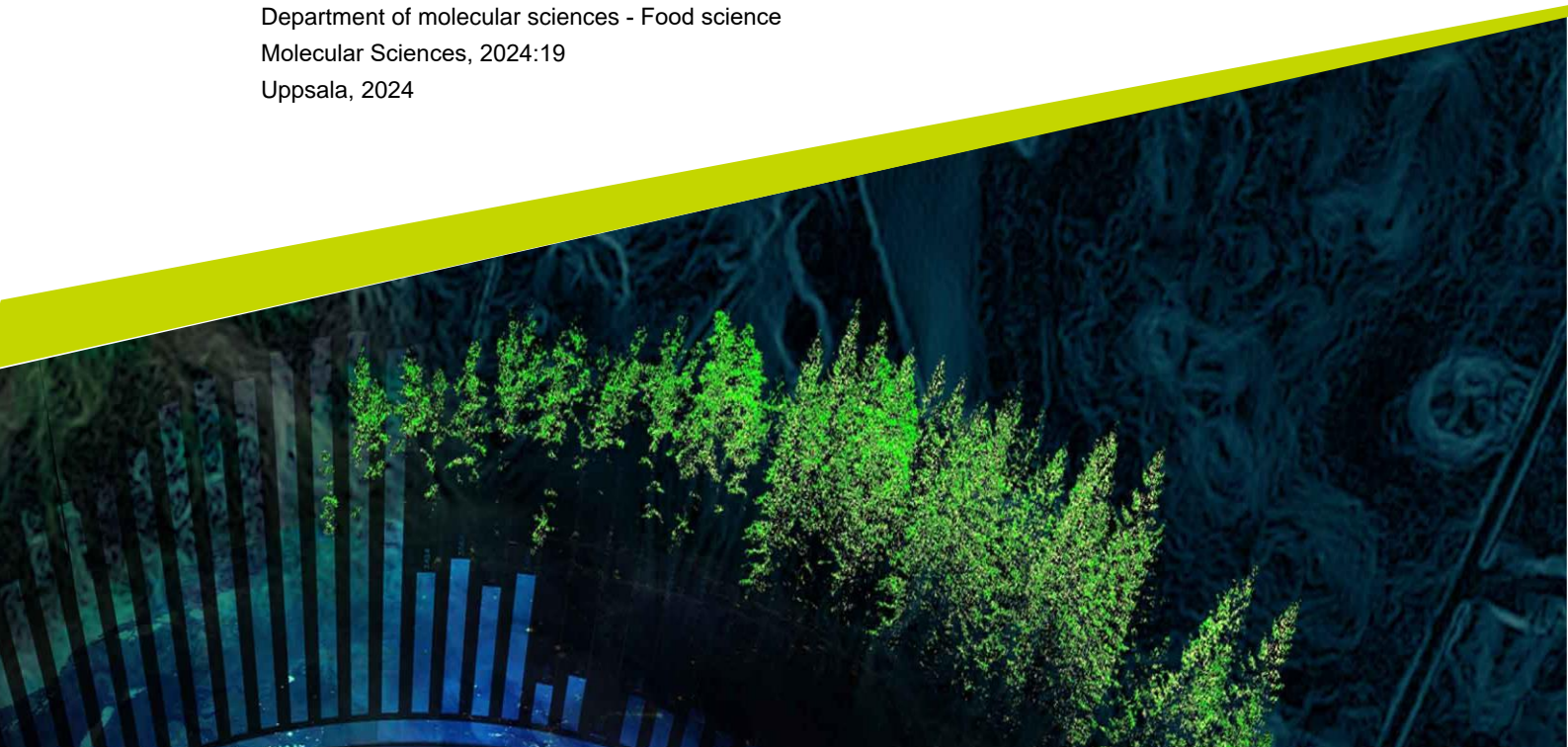


Microbial terroir in spontaneous fermented beer

Identification and quality aspects

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*Mikrobiell terroir i spontanjäst öl
-Identifikation och kvalitetsaspekter*

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Abstract

This thesis investigates the concept of microbial terroir in spontaneously fermented beers by comparing the microbial communities and flavor profiles of Belgian Lambic and American Coolship Ale (ACA). The study aims to determine if specific microorganisms tied to geographical locations influence the unique flavors of these beers and how barrel-aging affects their microflora and quality. Spontaneous fermentation relies on natural microflora present in the environment, resulting in unique and complex flavor profiles due to microbial succession through distinct fermentation phases: Enterobacteriaceae, main fermentation, acidification, and maturation.

Key methodologies include DNA amplification, high-throughput sequencing, gas chromatography-mass spectrometry (GC-MS), and gas chromatography-olfactometry, providing comprehensive insights into microbial diversity and volatile compound contributions. The research compares four studies: a study on Lambic from Cantillon's brewery, an industrial Lambic production, traditional Lambic from the Senne Valley, and ACA over three years.

Findings reveal common core microbiota, such as *S. cerevisiae*, *S. pastorianus*, *D. bruxellensis*, and *P. damnosus*, with variations suggesting microbial terroir. The study highlights significant differences in microbial compositions between Lambic and ACA, influenced by geographical and environmental factors. Insights from other fermented products like sourdough and Baijiu liquor further support the existence of microbial terroir.

This research underscores the complexity of spontaneous fermentation and its potential for producing unique beer flavors. Future studies should focus on controlled conditions and advanced microbial identification techniques to confirm microbial terroir and its impact on beer quality and flavor.

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Abbreviations

CF	Controlled fermentation
SF	Spontaneous fermentation
ACA	American Coolship Ale
LAB	Lactic acid bacteria
AAB	Acetic acid bacteria
GC-MS	Gas chromatography-mass spectrometry
GC-O	Gas chromatography-olfactometry
HPLC	High Pressure Liquid Chromatography
MALDI	
TOF-MS	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

1. Introduction

1.1 Background

The art of fermentation has been utilized throughout human history to preserve food and enhance desirable qualities, such as flavor and ethanol content. Initially, fermentation was a spontaneous process that relied on the microflora in the surrounding environment. However, today, it has evolved into a sophisticated and controlled process that employs specialized microorganisms to produce the desired product. In the brewing of beer and baking of bread, baker's yeast *Saccharomyces cerevisiae* (*S.cerevisiae*) is commonly used due to its ability to consume glucose and maltose, resulting in the production of carbon dioxide, ethanol, and other phenolic compounds that contribute to the flavor of the final product (Parapouli et al. 2020). Additionally, various strains of lactic acid bacteria (LAB), such as *Lactobacillus acidophilus*, are utilized to produce lactic acid when consuming lactose.

In modern times around the last 15 years, the beer and brewing culture has developed from a more streamlined flavor palate of lager beer to a more diverse flavor palate. Due to brewers looking back in history and the diversity of beer styles that are country or region-specific in their tasting profile and method. One country with old traditions and a unique approach to beer that has been kept around for a long time is the spontaneously fermented beers of Belgium such as Lambic beer—originating from the area of Pajottenland more specifically near the Senne River valley outside Brussels (Mittag 2014; Stoffelen 2022)

The principle of brewing this beer style that sets it apart from the standard brewing process is that the malt/water boil mixture called a *wort* is cooled down in open tanks. These tanks are called “koelschip”, doing it this way allows for the microflora in the brewery to interact with the wort. Everything from different wild yeast strains to LAB and acetic acid bacteria (AAB) will contribute to a unique flavor profile, quality, and experience of the beer with the downside of a slower fermentation.

1.2 The concept of microbial terroir

Microbial terroir as a concept is built on the word *terroir* used in the wine world. That summarizes the combined factors of a specific region's climate, soil, traditions, and to a certain extent microflora that contribute to a unique taste of the area (Van Leeuwen et al. 2020). When using the word terroir in a microbial context, the meaning of the word aims at microflora that is unique to a certain geographical area.

This would exclude beer with an added completely controlled fermentation factor such as a lab grown cultivated yeast strain or starter culture with LAB and AAB. Instead, studies of spontaneously fermented beers is the target for examining microbial terroir where a non-controlled inoculation of microbes is used, both by aerosol, barrel maturation, and sour mashing before the boil and hop addition (Peyer et al. 2017).

In the beer world, the most prominent scientific data found are on the spontaneously fermented beers of Belgium such as *Lambic*, *Flanders red ale*, and *Old Bruin*. Other known styles are the German *Berliner Weisse* and *Gose* as well as *American coolship ale (ACA)*. First, a comparison of controlled fermentation and spontaneous fermentation must be made, to point out key differences. To determine if microbial terroir influences the beer, a comparison between Lambic and ACA will be made, since their method is similar.

Not only to compare differences between Lambic and ACA but also the similarities between the Lambics brewed near the Senne River valley outside Brussels, Belgium. It could be possible that the variety in the microflora could even have developed into a unique combination in the specific brewery, called a house-flora. Where it's different but not necessarily apparently tied to the geographical location.

1.3 Potential for wild yeast and bacteria

The common standard microorganism used in alcoholic fermentation in the food industry is the yeast *S. cerevisiae*. Ever since *Emil Chr. Hansen* isolated the first pure yeast strain of “*S. carlsbergensis*” in 1883 yeasts have been well studied. *S. cerevisiae* was first isolated from the skin of grapes and the wild varieties are found everywhere in nature. Not only on the skin of grapes but also on the bark of trees, in the soil, on flowers, and even in the gastrointestinal tract of wasps, and flies. By cultivation, the wild *S. cerevisiae* has converted from surviving in low-nutrient environments and having slow fermentation to converting glucose and maltose to ethanol and carbon dioxide effectively (Kang et al. 2019)

Due to a more effective fermentation, interesting compounds released from the wild yeast varieties as secondary metabolites have to a certain degree got lost. Thus, spontaneous fermentation and mixed fermentation have the potential to unlock a wider spectrum of flavors for the beer consumer. Even though the historical Lambic beer from Belgium has been present for a long while, the popularity of sour ales comes from mixed fermentation beers such as the beer style *Berliner Weisse* or *Gose*. Which are beer styles using LAB as its only acidifier, the wort is pre-acidified before the yeast is added to perform the main fermentation. Due to its cleaner flavor profile and working well with the addition of fruits and berries leading its palate to soda/cider-like (Betancur et al. 2020; Sahu & Panda 2021; Hurt 2020)

Sour beers such as *Berliner Weisse* or *Gose* are nowadays brewed with a specific controlled inoculation of LAB via cultivated culture. These beers are not related to “microbial terroir” due to the absence of a specific geographical microflora. What sets spontaneously fermented beer apart, is the variety of LAB, and AAB yeast such as the *Brettanomyces sp.*, and to some degree, *Enterobacteriaceae* providing various secondary metabolites as aromas.

Typical flavors associated with spontaneously fermented beer such as Lambic are high acidity, tropical fruit, humid leather, clove, smoke, and spices. Fermenting with a controlled inoculation of microorganisms versus letting the wort inoculate spontaneously via aerosol has pros and cons regarding the final product. For that reason, the different methods must be compared to each other to understand the dynamics of the beer and the significance of spontaneous fermentation.

1.4 Thesis objective

The main objective of this thesis is to investigate the concept of microbial terroir in spontaneously fermented beers. By comparing the different microflora of Belgian Lambic and American Coolship ale. This thesis compares studies on microbial identification and their connection to flavor compounds through a literature review of available data on the topic.

The study aims to determine if the specific microorganisms tied to geographical locations influence the unique flavors of these beers and how barrel-aging affects microflora and beer quality. By utilizing advanced methods of microbial identification techniques such as DNA amplification, high-throughput sequencing, gas chromatography-mass spectrometry (GC-MC), and gas chromatography-olfactometry (GC-O). This review seeks to provide a detailed analysis of the microbial succession and its contribution to the flavor profiles of these beers, to try to determine if microbial terroir as a concept can be established.

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2. Literature review

2.1 Overview of fermentation

When comparing controlled fermentation (CF) and spontaneous fermentation (SF), key differences come from the microbial inoculation. Either the cooled wort is pitched with a certain yeast strain (CF), *S. cerevisiae* for ale, or *Saccharomyces pastorianus/Saccharomyces uvarum* for lager. These strains have been cultivated and specialized to efficiently convert pyruvate to ethanol and carbon dioxide via the Embden-Meyerhof-pathway (Nigam & Singh 2014; Stewart 2015). The fermentation is more predictable and leads to a consistent outcome in the final beer if the temperature and brewing method are accurate.

2.2 Fermentation vs. Spontaneous fermentation

Alternatively, there is the SF method that utilizes the microbes naturally present in the brewery's surroundings. By allowing the wort to inoculate with the microflora, a diverse array of yeasts and bacteria are incorporated, resulting in a unique beer with each brew. The SF method follows a multi-stage fermentation process where different microorganisms dominate the environment. Described as a succession as the beer medium transforms, influenced by factors like substrate availability, pH levels, and ethanol concentration. Four phases of succession have been identified in the SF of lambic beer. The *Enterobacteriaceae* phase, the main fermentation phase, the acidification phase, and the maturation phase (Van Oevelen et al. 1977; Verachtert & Iserentant 1995; Spitaels et al. 2014).

The variation in microflora and the respective diversity in biochemical pathways determines the flavor and time consumption to make the beer. That being the case, a general background of the more relevant microorganisms involved is necessary. For the (CF) *S. cerevisiae* and the (SF) LAB, AAB, and yeast typical for the lambic flavor the *Brettanomyces spp.*

2.3 Microbial succession in spontaneous fermented beer

Spontaneous fermentation means that no defined culture is added to the beverage or food item. Only the natural microflora is present in the matrix of the food/drink and in the surrounding environment that inoculates and starts fermenting.

The phrase spontaneous fermentation means in general the use of bacteria and yeasts found in aerosol and other ways to come in contact with the wort, without it being added in the first place (Maicas 2023; Mudoor Soorsh et al. 2023). Meaning that the result is less predictable therefore, blends are made in the case of lambic beer such as “*Gueuze*” where old, matured batches are blended with younger ones. What spontaneous fermentation adds is the microbial succession stages where different microorganisms dominate the medium where their primary metabolites and secondary metabolites give the unique flavor profile (Bongaerts et al. 2021; De Roos and & De Vuyst 2022).

The four stages of fermentation are described here according to a general pattern found in spontaneous fermented beer and sour beer overall described in these articles. To understand the result and comparison in the result section “microbial identification”.

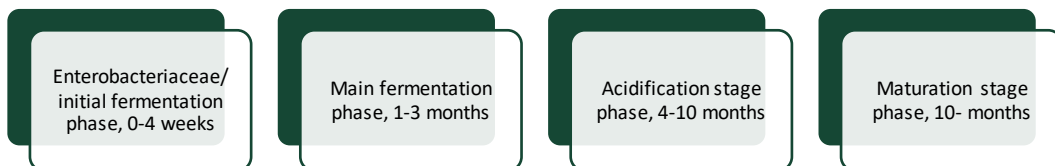


Figure 1. Description of microbial succession.

2.3.1 Enterobacteriaceae/ initial fermentation phase

During this stage, members of the Enterobacteriaceae family dominate with the presence of acetic acid bacteria and wild (oxidative) yeasts. This phase can last for anywhere between a week to a month but peaks around 1 week. During this first stage, small amounts of organic acids and ethanol are produced (De Roos et al. 2020; Piraine et al. 2021).

2.3.2 Main fermentation phase

In this phase, the dominant microbes are several species of *S. cerevisiae* due to the decrease in *Enterobacteriaceae* and oxidative yeast that produces competitive secondary metabolites such as carboxylic acid. In general, this stage lasts for about 9 weeks, where the maltose-positive yeasts *S. cerevisiae*, *S. pastorianus*, and *S. uvarum* are the main microorganisms responsible for the conversion of maltose and glucose to carbon dioxide and ethanol production together with other flavor compounds (Walker & Stewart 2016; Capece et al. 2018)

2.3.3 Acidification stage

Succeeding the yeasts is a larger variety of LAB and AAB that lowers the pH and introduces the characteristic acidity of spontaneously fermented beer. Typical bacteria found in the medium at this phase are *Pediococcus damnosus*, *Lactobacillus brevis*, *Acetobacter pasteurianus*, and *Acetobacter lambici* (De Roos & De Vuyst 2019; Su et al. 2020)

2.3.4 Maturation stage

Characterized by the presence of *Dekkera bruxellensis*, *Lactobacillus*, *Pediococcus damnosus*, and AAB. Starting around eight months after the fermentation initiation, during this stage the production of esters such as ethyl acetate and ethyl lactate is produced important for the final flavor.

2.4 Key microorganisms in fermentation

In the production of Spontaneous fermented beer, certain classes of microorganisms give the characteristic flavor. A combination of yeast and bacteria gives a complex mix of compounds affecting the final product. From the main fermenter *S. cerevisiae*, acidifiers such as LAB and AAB, to the characteristic yeasts for the maturation stage *Brettanomyces spp.*

2.4.1 Fermentation with *S. cerevisiae*

In the food industry, the standard yeast that is used is *S. cerevisiae* due to it tolerating higher ethanol levels, SO₂, and having a fast growth rate. It belongs to the genus *Saccharomyces* meaning “sugar fungus” and the species *cerevisiae*. It’s a single-cell microorganism, that reproduces itself via “budding” where the mother cell produces a daughter cell and a budding scar is left on the mother cell, a common way of asexual reproduction (Denoth Lippuner et al. 2014; Maicas 2020). *S. cerevisiae* is a facultative anaerobe and can grow in both the absence and in the presence of oxygen. By introducing oxygen into the beer wort, the yeast cells can synthesize sterols and fatty acids for their cell membrane. Leading to growth and cell division until all oxygen is consumed and the Embden-Meyerhof-pathway becomes the primary pathway instead (Rodriguez et al. 1985).

During fermentation, a wide range of secondary metabolites are produced due to the metabolism of the yeast cell. Here the temperature range serves an important role in the production of these compounds. The reason is that different compounds are synthesized depending on variations in environmental circumstances, and nutrition availability e.g. presence of fermentable carbons and nitrogen composition (Ruiz et al. 2010). Certain compounds are wanted, and some are not, the yeast can remove some of the impurities and unwanted compounds if enough time and temperature is provided.

The fermentation has two metabolic phases where different compounds are released. The primary and the secondary metabolism. During primary metabolism, the essential metabolic processes take place that are important for growth and cell division so that the cell can survive. Metabolic products are formed in this step including ethanol, acetic acid, acetaldehyde, and glycerol. Following the primary metabolism is the secondary metabolism where non-essential byproducts are formed including **terpenes, terpenoids, thiols, esters, carbonyls, and fusel alcohols** (Styger et al. 2011). These compounds play an important role in the beer flavor profile, but the most impact comes from the fusel alcohols and esters. The goal when brewing a beer is harmony in the entire beer medium referring to the

balance of sweetness, saltiness, bitterness, and alcohol spiciness to give a pleasant product for the consumer (Liu ChunFeng et al. 2008).

2.4.2 The importance of the *Brettanomyces sp* in spontaneous fermentation

A yeast family responsible for the typical flavors of lambic, often coming from the barrel itself where the SF is matured. Resistant to low pH and, to many detergents. Due to its endurance, it is almost impossible to get rid of in a brewery. Due to the porous structure of the wooden barrels, cleaning them is very difficult meaning that *Brettanomyces* can survive every time after a new batch of lambic. The succession in microbial flora is directly linked to substrate availability. When maltose and glucose disappear in the medium, the *S. cerevisiae* population declines. Following the main fermentation stage is the maturation stage where *Brettanomyces* the LAB *Pediococcus* increases in quantity. The reason for this shift in the microflora is due to the two types of microorganisms above that can metabolize dextrin, this also relates to the further acidification of the beer medium.

The most prominent *Brettanomyces* species is *Dekkera bruxellensis* found in all lambic beers. Typical flavors associated with “brett” are a certain combination of ethyl esters, phenolic compounds, and fatty acids that give a tropical fruit, humid leather, clove, smoke, spice, and an animalic taste (Spitaels et al. 2014). *Brettanomyces* are often considered a spoilage microorganism that gives off-flavors in wine and other fermented foods. It's only really wanted in Belgian-style beers such as lambic, American coolship ale, and the Belgian Trappist beer *Orval* (Smith & Divol 2016).

2.4.3 Lactic acid bacteria (LAB)

A large group of facultatively anaerobic, gram-positive, non-spore-forming bacteria with tolerance for low pH environment (Caggianiello et al. 2016). They play an important role in human microbiota in the intestinal helping with digestion and competing with pathogenic bacteria (Zaky et al. 2021). LAB ferments carbohydrates to receive energy and produces numerous metabolites during fermentation. Secondary metabolites such as alcohols, aldehydes, ketones, esters, organic acids, phenolic and heterocyclic compounds (Dongmo et al. 2016; Şanlıer et al. 2019). The most common genera isolated in sour beers such as *Lambic*, *ACA*, *Berliner Weisse*, and *Gose* are *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Pediococcus*.

In *Berliner Weisse*, the dominant species found are *Lactobacillus brevis* and *Lactobacillus delbrueckii*, while *Pediococcus* strains are more common in *Lambic* and *Gueuze* (Preedy 2011; Spitaels et al. 2015b). In general, LAB are sensitive to the active compounds in hops such as the iso- α -acids and iso- β -acids, consequently, hops with a lower quantity of α -acids and β -acids are used when producing sour beers, the most resistant variety of LAB is *L. brevis* (Haakensen et al. 2007; Suzuki 2015). LAB can produce many secondary metabolites as mentioned above, however the production of lactic acid is its main contributor to the flavor palate.

2.4.4 Acetic acid bacteria (AAB)

AAB serves the role of acidifier in the beer medium together with the LAB to create the high acidity associated with beer styles such as *Lambic* and *ACA* and to produce vinegar.

They are obligate aerobic bacteria, so they need oxygen to survive, AAB oxidizes glucose and ethanol into acetic acid and gluconic acid. Common isolates of AAB in *Lambic* beer and other fermented foods are *Acetobacter orientalis*, *Acetobacter pasteurianus*, *Acetobacter lambici*, and *Gluconobacter cerevisiae* according to (De Roos et al. 2018; De Roos & De Vuyst 2018; Zheng et al. 2018). The detection of the AAB has varied through studies due to samples being taken through the cork plug of the barrel, which is located just above the bottom of the barrel. Since AAB need oxygen, they are likely in higher concentration where the air and liquid meet in the barrel closer to the wood.

2.5 Secondary metabolites spontaneously fermented beer

As a result of the various microorganisms found in the beer, an extensive list of secondary metabolites can be found. Certain ones play major roles in the flavor and have a low flavor threshold, and some are just present in the medium without any significant impact. Compounds that play an important role in the final product of the spontaneously fermented beer are **esters, fusel/higher alcohols, carbonyls, aldehydes, acids, and thiols (sulfur)**. Usually, there are terpenes and terpenoids in beer coming from the hops and the interaction between hops and yeast. This is not the case in Spontaneous fermented beer, where over-aged hops are often used to not get too much bitterness and compounds interfering with the microflora (Aguiar et al. 2022). Regarding the secondary metabolites, most research is conducted on *S. cerevisiae* and *Brettanomyces spp* when it comes to directly linked flavor compounds.

A study was conducted where *Gueuze* beers from different aging stages were analyzed via GC-O to detect aroma compounds. To match the detection by the GCO, an experienced tasting panel made up of 3 persons (1 female and 2 males) also tried the beers according to a protocol from (Ferreira et al. 2003). By using a testing panel, compounds that are irrelevant to the flavor experience can be excluded from the result regarding the beer quality. Twenty compounds were detected by the GC-O, yet none of the compounds was detected by everyone in the panel. The compound with the highest detection rate was ethyl hexanoate and only 60% detected propanoic acid, 2-methyl-1-butanol, isoamyl alcohol, and decanal (Witrick et al. 2020).

The vast array of aromas in spontaneously fermented beers is due to the diversity of microorganisms changing the medium and releasing secondary metabolites. Typical acids are lactic acid and acetic acid produce by LAB and AAB that generally drop the pH to 3.3-3.9 according to (Tonsmeire & Cilurzo 2014; Peyer 2017) compared to commercial beers at 3.7-4.8 (Olaniran et al. 2017). This set it apart from other beer styles where the most flavor comes from the malt and the hops. A wide variety of compounds are synthesized during the fermentation and maturation process, the most important ones for flavor according to (Meilgaard 1982; Langos et al. 2013) the fusel/higher alcohols, esters, organic acids, dimethyl sulfide, and diacetyl. Different compounds also have different flavor thresholds, thus small amounts of certain ones are more noticeable in the beer medium (James & Stahl 2014). The following part of this chapter briefly describes the most common occurring flavor compounds associated with spontaneously fermented beer and is complemented by **Table 1** listing the common compounds.

Esters

A group of flavor compounds associated with a “fruity aroma” in the beer flavor profile. Esters are formed via an enzymatically catalyzed condensation of acyl CoA and higher alcohol (fusel alcohols)(Ploier et al. 2015).

In the beer flavor profile ethyl acetate and isoamyl acetate are the most impactful. Low-content esters such as ethyl octanoate, isobutyl acetate, ethyl butyrate, and phenylethyl, acetate have a lower contribution but can have some impact.

Fusel alcohols

The fusel alcohols are a left-over product from when the yeast absorbs amino acids from the beer wort to utilize its amino group in its structure. After this, only an α -keto acid is left to go into a reaction process which is irreversible to become a fusel alcohol (Pires et al. 2014). Except for giving the beer flavor on their own, fusel alcohols are also important for the formation of esters (Dack et al. 2017). Commonly among the fusel alcohols, amyl alcohol, isobutanol, propanol, methyl butanol, and phenyl alcohol are found.

Carbonyls

A category of compounds that are generally in lower concentration but can have a big impact on the beer flavor and mouthfeel. The most common one is diacetyl(2,3-butanedione) and 2,3-pentadione which are formed during primary fermentation as a by-product of the synthesis of the amino acids valine and isoleucine. The presence of diacetyl is a sign of the maturation of the beer and successful fermentation. Flavors associated with diacetyl are butter/toffee, which might be wanted to a certain degree in scotch ales, and stouts. Most of the time it's not wanted, especially not in lager styles of beer, except for the beer *Pilsner Urquell* from the Czech Republic, where it's a trademarked characteristic (Krogerus & Gibson 2013).

Aldehydes

Compounds formed through the oxidation of primary alcohols. Aldehydes can be synthesized in other ways also, e.g. the formation of acetaldehyde (green apple aroma) by the yeast as an intermediate in the glycolytic pathway in the beginning of the fermentation. The synthesis of acetaldehyde is also initiated by yeast of low quality or deteriorating microorganisms (Jackowitz et al. 2011; Shin & Lee 2019)

Thiols

The sulfur-containing analog to alcohols is found in the plant's raw material, some can be transferred directly to the wort, for example, the ones found in hops and grapes. But the majority comes from carbon-sulfur lyases such as β -lyases in *S. cerevisiae* that react with glutathionylated or cysteinylated precursors found in malt (Svedlund et al. 2022). These sulfur compounds have a strong aroma in very low concentrations and some of them can give an onion, skunk-like aroma.

Phenols

In terms of the flavor characteristics of spontaneously fermented beer, the main characteristic is the leathery, medicinal, mousy, clove, and barnyard flavor produced by the *Brettanomyces spp.* This is due to an enzyme that the *Brettanomyces* yeast contains, with the name vinyl phenol reductase. By catalyzing the transformation of vinyl phenols and guaiacols into their ethyl forms 4ethylphenol(4EP) and 4-ethylguaiacol(4EG) (Steensels et al. 2015; Holt et al. 2018).

Table 1. Common flavor compounds in spontaneously fermented beer.

Esters:
<i>Ethyl acetate: fruity</i>
<i>Isoamyl acetate: banana and solvent</i>
<i>Ethyl octanoate: brandy aroma, sweet</i>
<i>Isobutyl acetate: fruit, solvent</i>
<i>Phenylethyl acetate: rose fragrance</i>
<i>Ethyl butyrate: papaya, cream, pineapple.</i>
Fusel/higher alcohols:
<i>Amyl alcohol, isobutanol, and propanol: give a solvent-like taste and warm feeling in the mouth, that typical “alcohol-flavor”.</i>
<i>Methyl butanol: medicinal, banana flavor</i>
<i>Phenyl alcohol: rose, sweet perfume.</i>
Carbonyls:
<i>Diacetyl(2,3-butanedion): Butter/ butterscotch</i>
<i>2,3-pentadione: toffee</i>
Organic acids:
<i>Lactic acid: clean tartness, sourness</i>
<i>Acetic acid: sour, astringent, pungent sourness, vinegar.</i>
<i>Isovaleric: rancid cheese, common in food odor</i>
<i>Hexanoic: Fatty, cheesy, waxy, and animalic (barnyard animals)</i>
<i>Valeric acid: pungent, sour milk, tobacco, and fruitiness.</i>
<i>Octanoic acid: wax. Soap. Goat, musty, rancid, and fruity</i>
<i>Dimethyl sulfide (Enterobacteriaceae): Wild radish, sharp, green, cabbage-like, repulsive odor</i>
Aldehydes:
<i>Acetaldehyde: green apple</i>
<i>Decanal: floral, fresh, citrus.</i>
Thiols:
<i>3-mercaptohexanol (3MH): grapefruit (malt)</i>
<i>4-mercapto-4-methylpentan-2-one (4MMP): black currant</i>
<i>3-sulfanyl-4-methyl-pentan-1-ol (3S4MP): grapefruit and rhubarb.</i>
Phenols:
<i>4-ethylphenol: musky</i>
<i>4-ethylguaiacol: soy sauce</i>

2.6 Analytical methods for assessment of microbial terroir

To identify and compare the microbial communities in the spontaneously fermented beers, accurate methods and techniques must be employed. The combination of these methods ensures a comprehensive understanding of the microbial dynamics and how they contribute to the beer flavor profile.

DNA Amplification

These techniques such as polymerase chain reaction (PCR) is utilized to detect and quantify specific microorganisms present in the beer samples. The method involves extraction of DNA from the samples, amplifying target regions using specific primers and analyzing the amplified products through sequencing or gel electrophoresis (Sathiamoorthy et al. 2018)

High-Throughput Sequencing

High-throughput sequencing, such as Illumina sequencing, is used to characterize the microbial diversity in the beer samples. By providing the detailed profile of microbial communities by sequencing the DNA of all microorganisms present. The data generated is analyzed using bioinformatics tools to identify and quantify the different species (Caporaso et al. 2012).

Shotgun Genomics:

The method involves sequencing the entire genome of microorganisms present in a sample. Shotgun genomic provides comprehensive data on the genetic composition, allowing for a detailed analysis of the microbial community and its functional potential (Quince et al. 2017)

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS):

This method is used for rapid identification of microorganisms based on the unique protein fingerprints of different species. It involves ionizing the proteins and measuring their mass-to-charge ratio, providing a quick and accurate identification of microbial species present in the samples (Tran et al. 2015)

Gas Chromatography-Mass Spectrometry (GC-MS)

This method is used to analyze the volatile compound responsible for the flavor profiles of beers. The technique involves separating the compounds in a gas chromatograph and identifying them using a mass spectrometer. GC-MS provides a detailed chemical profile of the beer and highlighting the contribution of different microorganisms to the flavor (Adams 2017).

GC-MS-Olfactory

GC-MS-Olfactory combines GC-MS with olfactory detection to identify aromatic compounds. When compounds are separated in the gas chromatograph, they are the same time detected by a human nose and mass spectrometer. The combination of these methods helps to correlate the specific volatile compounds with sensory perceptions, providing insight into the flavor contribution of the microorganism's present (Song & Liu 2018).

High-Performance Liquid Chromatography (HPLC):

HPLC is used to separate, identify, and quantify non-volatile compounds in the beer samples. The technique involves passing a liquid sample through a column filled with a solid adsorbent material, allowing for the separation of compounds based on the interactions with the adsorbent. HPLC complements the data obtained from GC-MS to give detailed information about the beer's chemical composition (Snyder et al. 2011).

3. Results and discussion

3.1 Microbial identification

When the principles of CF and SF and their differences have been described, a direct comparison between different SF can be made. By identifying and comparing the microbial floras of ACA and Belgian Lambic Beer. This comparison will lay the foundation for what can be described as microbial terroir in spontaneously fermented beer. Also to shed some light on how the barrel aging affects the beer's microbial flora and the quality of the beer.

The four studies seen in **Table 2** were chosen for comparison due to their similar microbial identification method, and DNA amplification. They will be examined through the previously described microbial succession in spontaneously fermented beer. To get a representative comparison between the beers, 1 year of fermentation was chosen to get an even comparison between the studies. Even though some of the studies span longer than 1 year, even up to 3 years, the 1-year mark give a clear picture of all four phases. To encapsulate the Enterobacteriaceae phase, the main fermentation phase, the acidification phase, and the maturation phase.

Three lambics are compared with one ACA, to see if significant differences can point to the phenomenon of microbial terroir. After the comparison, the key microflora will be compared to studies regarding the aromas found in spontaneously fermented beer. Most studies are conducted on *Gueuze* beer which is a blend of different ages and barrels of lambic beer. Because it gives an entire spectrum of the aroma compounds, accounting for the variabilities in the barrels.

Table 2. Studies for comparison of spontaneously fermented beer.

Study 1: This first study compared two casks of Lambic beer from Cantillon's brewery (http:// www.cantillon.be), in Brussels (Spitaels et al. 2014).
Study 2: A study produced industrial lambic beer using bigger wooden barrels and cooling systems to allow year-round production (Spitaels et al. 2015a).
Study 3: A study was done on the traditional produce lambic from the Senne Valley outside Brussels (De Roos & De Vuyst 2019).
Study 4: For 3 years 8 batches of American coolship beer were sampled to identify the microbial community. Samples were taken from different barrels to get a representative span of the entire fermentation (Bokulich et al. 2012).

By looking at the first phase of the SF seen in **Table 3**, the one standing out is the industrial lambic from **Study 2**, where the *Enterobacteriaceae* was non-existent due to pre-acidifying the wort. Only the yeast *Hanseniaspora uvarum* which was the main fermenter until month 2 together with *Pichia fermentans*. However, *Hanseniaspora uvarum* was also a main yeast in **Study 3**. The other three studies had a clear community of *Enterobacteriaceae* where *Klebsiella oxytoca* was the only one found in all three studies. A common theme is the appearance of the main fermentation yeast after weeks 1-2, either *S. cerevisiae*, *S. pastorianus*, or *S. bayanus*.

In the main fermentation phase, *S. cerevisiae*, *S. pastorianus*, or *S. bayanus* are the main fermenting yeast in the studies, whereas **Study 2** expects *P. fermentans* and **Study 3** has *Saccharomyces kudriavzevii* as the main fermenting yeasts as well. Regarding bacterial flora during the main fermentation phase, it varies a lot between the studies. Common themes are the presence of AAB (*Acetobacter pasteurianus* and *A. lambici*) and LAB (*Lactobacillus brevis*, *Lactobacillus lindneri* and *Lactococcus lactis*) with **Studies 2 and 4** showing the most diversity. In three of the studies, *Pediococcus* spp especially *P. damnosus* makes its first appearance, which will later dominate the bacterial community in all studies. **Study 2** is the only one where *Gluconobacter* is found in the form of *G. cerevisiae* and *G. cerinus*.

During the acidification phase, the main yeast present in all studies is *D. bruxellensis*. **Study 1** and **3** show a wider range of main yeast varieties, where **Study 1** has the *Debaromyces* species as main fermenters from month 6, such as *D. marama* and *D. hansenii* together with some minor yeast *Naumovia castellii*,

Priceomyces carsonii, and *Candida friedricii*. **Study 3** has *Pichia membranifaciens* as a main fermenter with *D. bruxellensis*. The main acidifying bacteria is dominantly *P. damnosus* in all studies with some *Acetobacter fabarum* in **study 2**. Once again **Study 4** of the American coolship ale shows a wide variety of LAB (*pediococcus spp*, *L. lactis*, *Lactococcus garviae*, *Lactococcus spp*, and some *Leucoccus mesenteroides*).

The final phase, the maturation phase show a high concentration of *D. bruxellensis* as expected, but **Study 1** also had *D. hansenii* and **Study 3** had *Pichia membranifaciens* as primary yeasts. *P. damnosus* was dominating in the bacterial community with some *A. pasteurianus* in **Study 3** and *L. lindneri* in **Study 4**. More specifics can be found in **Table 3**.

Table 3. Microbial succession between studies

Yeast and bacteria are in falling order from highest concentration, *indicates main fermenters and yellow coloring indicates if microflora repeats.

	Study 1	Study 2	Study 3	Study 4
Enterobacteriaceae phase 0-4 weeks	<i>S. cerevisiae</i> ,* <i>S. pastorianus</i> * <i>E. hormaechei</i> <i>E. kobei</i> <i>H. paralvei</i> <i>K. oxytoca</i>	<i>H. uvarum</i> * <i>P. fermentans</i>	<i>H. uvarum</i> * <i>S. cerevisiae</i> * <i>kazachstania</i> species <i>K. oxytoca</i> * <i>K. variicola</i> * <i>A. orientalis</i>	<i>S. cerevisiae</i> / <i>S. bayannus</i> * <i>K. oxytoca</i> * <i>E. agglomerans</i> <i>L. brevis</i>
Main fermentation phase 1-3 months	<i>S. cerevisiae</i> * <i>D. hansenii</i> <i>S. pastorianus</i> * <i>P. damnosus</i> * <i>A. lambici</i>	<i>S. pastorianus</i> * <i>P. fermentans</i> * <i>S. cerevisiae</i> <i>G. cerinus</i> <i>A. lambici</i> <i>P. damnosus</i> <i>G. cerevisiae</i>	<i>S. cerevisiae</i> * <i>S. kudriavzevii</i> <i>A. pasteurianus</i>	<i>S. cerevisiae</i> / <i>S. bayannus</i> * <i>Pediococcus</i> * <i>L. brevis</i> <i>L. lindneri</i> <i>Lc. lactis</i>
Acidification phase 4-10 months	<u>Month 6</u> <i>D. bruxellensis</i> * <i>D. marama</i> <i>N. castellii</i> . <u>Month 9</u> <i>D. hansenii</i> <i>P. carsonii</i> <i>C. friedricii</i> <i>P. damnosus</i> * (only bacteria)	<i>D. bruxellensis</i> * <i>A. fabarum</i> <i>P. damnosus</i> *	<i>P. membranifaciens</i> * <i>D. bruxellensis</i> * (main) <i>Ogateae</i> <i>Candida</i> . <i>P. damnosus</i> *	<i>D. bruxellensis</i> * <i>Pediococcus spp</i> * <i>Lc. lactis</i> , <i>L. garviae</i> , <i>Lactococcus spp</i> , <i>Leuc</i> . <i>Mesenteroids</i> . <i>Enterobacteriaceae</i>
Maturation phase 10- months	<i>D. bruxellensis</i> <i>D. hansenii</i> , <i>P. carsonii</i> <i>C. friedricii</i> . <i>P. damnosus</i>	<i>D. bruxellensis</i> * <i>P. damnosus</i> *	<i>D. bruxellensis</i> <i>P. membranifaciens</i> (main) <i>K. bulderei</i> <i>D. custerianus</i> <i>P. damnosus</i> <i>A. pasteurianus</i>	<i>D. bruxellensis</i> (main) <i>C. krusei</i> <i>Pediococcus</i> <i>Lactobacillus</i> , <i>L.lindneri</i> <i>bacillales</i>

3.2 Linkage to microbial terroir

Microbial terroir can only be determined if the microflora can be tied to the geographical location and give certain flavor characteristics. By looking at the four studies from the previous chapter, there is certainly potential for microbial flavor uniqueness. Some key microorganisms were seen in all the samples such as the main fermenters *S. cerevisiae*, *S. pastorianus*, *D. bruxellensis*, and *P. damnosus*. Another microorganism that was seen in more than one study was the enterobacteria. *K. oxytoca* and the AAB *A. lambici*. The rest are unique to the separate studies with various yeast, AAB, and LAB. The ACA didn't have AAB and a much lower quantity of yeast variations during the different fermentation phases.

Most data regarding terroir are found in the wine sector. A study published in the Australian Journal of grape and wine research 2023, investigated differences in variety in microbial communities in the PDO Nemea zone in Greece. The study was conducted on three vineyards in the area with some different attributes to them. Using classic microbial analysis and metataxonomic to prove the variability of microbes. The control was on soil, grapes, and wine during both controlled and spontaneous fermentation. Most diversification was found among the bacterial communities and less in the yeast/fungi. It was also found that the regional geographical effect was more prominent among samples of grapes and wine and not significantly in the soil (Kazou et al. 2023).

Studies conducted on microbial diversity among yeast species on wine grapes in Chile showed that the humidity in the area greatly affected the yeast diversity. Higher relative humidity showed a higher quantity and variety of yeast such as *Hanseniaspora* but affected *Saccharomyces* negatively (Jara et al. 2016). So, a possible relation to the time of the year and its weather conditions can affect the microflora in the brewery and beer. Microbial diversity has been seen in several studies even comparing different grapes and terroir, comparing spontaneous fermentation and completely controlled fermentation. This showed that the terroir had an impact in the initial stages of the fermentation but not as the fermentation progressed where the environment for the microbes became harsher.

3.3 Insight from other studies

A study with shotgun genomics compared 17 different sourdough starters from different countries, including France, the UK, the US, and Belgium. These were also divided into subgroups of origin: household, artisan bakery, and Lambic brewery as well as grains: rye, spelt, wheat, buckwheat, kamut, whole wheat, and wheat and Lambic. It was found that the sourdough from the same location had a similar microbial makeup independent of the type of flour and geographical region (Comasio et al. 2020). This was also confirmed when a study looked at sourdoughs from four artisan bakeries in Italy, where a dominant house flora of LAB and yeast was found on bakery equipment as well as in the specific sourdoughs (Minervini et al. 2015).

The Belgian sourdoughs stood out for their higher diversity of bacteria and yeast, remarkably the ones coming from the lambic brewery were the only sourdoughs with AAB in them. The AAB in the sourdough can come from cross-contamination from the Lambic brewing process but also be connected to fruit flies which carries AAB (Matsushita et al. 2016). Yeasts isolated from the same sourdoughs were *D. bruxellensis*, *D. anomala* and *P. membranifaciens* associated with Lambic brewing (Comasio et al. 2020). Factors affecting spontaneous fermentation both in sourdough and Lambic are also related to the raw material used or what has been introduced to the production facility/ brewery such as apple flowers, hawthorn berries, pomegranate, and even mother of vinegar (Ripari et al. 2016).

When looking at Chinese *Baijiu* liquor belonging to different aroma types, from 9 different locations between a latitude range of 27°N to 37°N. It was shown that the latitude affected the fungal community more than the longitude. The bacterial community had a weak correlation with longitude and was not geographically dependent to the same degree as the fungi. Differences in aroma compounds between the aroma types of the *Baijiu* liquor were mainly attributed to the cometabolism between the fungi and bacteria. Using GC-MS was used to measure the metabolite profiles of the liqueurs, of the 471 volatile compounds only 56 were shared among the aroma types, esters had the highest concentration (Tan et al. 2022).

3.4 Implication of Spontaneous Fermentation

The linkage between weather conditions, microflora, and microbial activity is well known. This was noticeable when a study looked at the microbial diversity in raw milk over 12 months, once again stating the temperature and humidity as the main factors determining the microflora composition. e.g. a higher concentration of *Firmicutes* was related to high temperature and low temperature was a higher concentration of *Actinobacteria*. At the same time, a higher *Proteobacteria* concentration was correlated with high humidity and *Bacteroidetes* with low humidity. Similar to some of the studies of spontaneously fermented beers compared earlier, the LAB was not present in a regular pattern but showed up irregularly over the fermentation process (Quigley et al. 2011; Li et al. 2018).

Just as the variation of microorganisms varies through the seasons, it also determines the rate of metabolic activity giving rise to new interactions and flavors in the fermenting medium. To a certain extent, the significance also comes down to the brewing method, shown in e.g. the industrial Lambic from **study 2** where the Enterobacteriaceae phases were absent when the wort was pre-acidified. Shows that spontaneous fermentation and its link to microbial terroir is complex and a wide variety of factors such as temperature, humidity, raw material, method, and location affect the microflora.

4. Conclusions and further research

4.1 Conclusions

This thesis explores the concept of microbial terroir in spontaneously fermented beer. Looking specifically at Belgian Lambic and American Coolship Ale (ACA), by a comprehensive analysis of the microbial communities and their involvement in flavor characteristics. Shedding light on the potential influence of the geographical location and environmental factors to give the beer a unique flavor.

Key findings in the comparison of Belgian Lambic and ACA are their core microbiota of *S. cerevisiae*, *S. pastorianus*, *D. bruxellensis*, and *P. damnosus*. Nonetheless, notable variations of microorganisms were found in the two beer types, showing a unique combination of yeasts, lactic acid bacteria (LAB), and acetic acid bacteria (AAB) contributing to possible unique flavor compounds being released, suggesting a potential microbial terroir. The ACA samples displayed and lack of AAB and fewer main fermenting yeast, differentiating it from the other samples of Belgian Lambic. However, due to the lack of more studies on ACA, a conclusion can't be made. Also, the study on ACA was conducted on several barrels, where the main features could be found between them.

The study also demonstrates the importance of microbial succession in spontaneous fermentation. Where the different stages—initial fermentation, main fermentation, acidification, and—maturation to a great extent shape the beer's flavor profile. Setting spontaneously fermented beer apart from beer produced via controlled fermentation.

4.2 Further research

To further the research in microbial terroir, a variety of approach can be focused on to more thorough analysis of the microbial diversity of spontaneously fermented beer and how it affects the flavor.

By using the analytic techniques presented in the methods section to conduct controlled studies using newly made oak barrels from the same producer and with similar seasonal conditions to isolate the impact of the geographical flora, to tie it to a specific location. Advanced microbial identification techniques are essential such as DNA amplification, shotgun genomics, and MALDI-TOF to get a more accurate and comprehensive identification of microbial communities and a deeper insight into the diversity and dynamics of the microflora.

Combining the advanced microbial identification techniques with the analysis of volatile and non-volatile compounds using GC-O and HPLC can aid in the correlation establishment between specific microorganisms and the contribution to the beer flavor. This should also be paired with the trained sensory panel to validate the impact of microbial terroir on the flavor and if it suits consumers' preferences.

To get a more wide and diverse comparison more regions should be involved in these studies and factor in the effect of the weather conditions, temperature, humidity, and raw materials to see how it affects the dynamics and microflora.

By addressing these areas, future research can provide more definitive evidence of microbial terroir and its significant impact on the flavor and quality of spontaneously fermented beers. This understanding can enhance brewing practices, promote traditional beer styles, and inspire innovations in the craft beer industry.

5. References

- Adams, R.P. (2017). Identification of essential oil components by gas chromatography/mass spectrometry. 5 online ed. *Gruver, TX USA: Texensis Publishing*,
- Betancur, M.I., Motoki, K., Spence, C. & Velasco, C. (2020). Factors influencing the choice of beer: A review. *Food Research International*, 137, 109367. <https://doi.org/10.1016/j.foodres.2020.109367>
- Bokulich, N.A., Bamforth, C.W. & Mills, D.A. (2012). Brewhouse-Resident Microbiota Are Responsible for Multi-Stage Fermentation of American Coolship Ale. *PLOS ONE*, 7 (4), e35507. <https://doi.org/10.1371/journal.pone.0035507>
- Bongaerts, D., De Roos, J. & De Vuyst, L. (2021). Technological and Environmental Features Determine the Uniqueness of the Lambic Beer Microbiota and Production Process. Björkroth, J. (red.) (Björkroth, J., red.) *Applied and Environmental Microbiology*, 87 (18), e00612-21. <https://doi.org/10.1128/AEM.00612-21>
- Caggianiello, G., Kleerebezem, M. & Spano, G. (2016). Exopolysaccharides produced by lactic acid bacteria: from health-promoting benefits to stress tolerance mechanisms. *Applied Microbiology and Biotechnology*, 100 (9), 3877–3886. <https://doi.org/10.1007/s00253-016-7471-2>
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G. & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, 6 (8), 1621–1624. <https://doi.org/10.1038/ismej.2012.8>
- Comasio, A., Verce, M., Van Kerrebroeck, S. & De Vuyst, L. (2020). Diverse Microbial Composition of Sourdoughs From Different Origins. *Frontiers in Microbiology*, 11. <https://doi.org/10.3389/fmicb.2020.01212>
- Dack, R.E., Black, G.W. & Koutsidis, G. (2017). The effect of Maillard reaction products and yeast strain on the synthesis of key higher alcohols and esters in beer fermentations. *Food chemistry*, 232, 595–601
- De Roos and, J. & De Vuyst, L. (2022). Lambic Beer, A Unique Blend of Tradition and Good Microorganisms. I: *Good Microbes in Medicine, Food Production, Biotechnology, Bioremediation, and Agriculture*. John Wiley & Sons, Ltd. 225–235. <https://doi.org/10.1002/9781119762621.ch18>
- De Roos, J. & De Vuyst, L. (2018). Acetic acid bacteria in fermented foods and beverages. *Current Opinion in Biotechnology*, 49, 115–119. <https://doi.org/10.1016/j.copbio.2017.08.007>

- De Roos, J. & De Vuyst, L. (2019). Microbial acidification, alcoholization, and aroma production during spontaneous lambic beer production. *Journal of the Science of Food and Agriculture*, 99 (1), 25–38. <https://doi.org/10.1002/jsfa.9291>
- De Roos, J., Verce, M., Aerts, M., Vandamme, P. & De Vuyst, L. (2018). Temporal and Spatial Distribution of the Acetic Acid Bacterium Communities throughout the Wooden Casks Used for the Fermentation and Maturation of Lambic Beer Underlines Their Functional Role. *Applied and Environmental Microbiology*, 84 (7), e02846-17. <https://doi.org/10.1128/AEM.02846-17>
- Denoth Lippuner, A., Julou, T. & Barral, Y. (2014). Budding yeast as a model organism to study the effects of age. *FEMS Microbiology Reviews*, 38 (2), 300–325. <https://doi.org/10.1111/1574-6976.12060>
- Dongmo, S.N., Procopio, S., Sacher, B. & Becker, T. (2016). Flavor of lactic acid fermented malt based beverages: Current status and perspectives. *Trends in Food Science & Technology*, 54, 37–51
- Ferreira, V., Pet'ka, J., Aznar, M. & Cacho, J. (2003). Quantitative gas chromatography–olfactometry. Analytical characteristics of a panel of judges using a simple quantitative scale as gas chromatography detector. *Journal of Chromatography A*, 1002 (1), 169–178. [https://doi.org/10.1016/S0021-9673\(03\)00738-6](https://doi.org/10.1016/S0021-9673(03)00738-6)
- Haakensen, M.C., Butt, L., Chaban, B., Deneer, H., Ziola, B. & Dowgiert, T. (2007). horA-specific real-time PCR for detection of beer-spoilage lactic acid bacteria. *Journal of the American Society of Brewing Chemists*, 65 (3), 157–165
- Holt, S., Mukherjee, V., Lievens, B., Verstrepen, K.J. & Thevelein, J.M. (2018). Bioflavoring by non-conventional yeasts in sequential beer fermentations. *Food Microbiology*, 72, 55–66
- Hurt, J. (2020). *Beer Drinkers Are Growing Sweet On Sour Beers*. *Forbes*. <https://www.forbes.com/sites/jeanettehurt/2020/03/11/beer-drinkers-aregrowing-sweet-on-sour-beers/> [2024-05-09]
- Jackowetz, J.N., Dierschke, S. & Mira de Orduña, R. (2011). Multifactorial analysis of acetaldehyde kinetics during alcoholic fermentation by *Saccharomyces cerevisiae*. *Food Research International*, 44 (1), 310–316. <https://doi.org/10.1016/j.foodres.2010.10.014>
- James, N. & Stahl, U. (2014). Amino Acid Permeases and their Influence on Flavour Compounds in Beer. 67
- Jara, C., Laurie, V.F., Mas, A. & Romero, J. (2016). Microbial Terroir in Chilean Valleys: Diversity of Non-conventional Yeast. *Frontiers in Microbiology*, 7. <https://doi.org/10.3389/fmicb.2016.00663>
- Kang, K., Bergdahl, B., Machado, D., Dato, L., Han, T.-L., Li, J., Villas-Boas, S., Herrgård, M.J., Förster, J. & Panagiotou, G. (2019). Linking genetic, metabolic, and phenotypic diversity among *Saccharomyces cerevisiae* strains using multi-omics associations. *Gigascience*, 8 (4), giz015
- Krogerus, K. & Gibson, B.R. (2013). 125th Anniversary Review: Diacetyl and its control during brewery fermentation: Diacetyl and its control during brewery fermentation. *Journal of the Institute of Brewing*, n/a-n/a. <https://doi.org/10.1002/jib.84>
- Langos, D., Granvogl, M. & Schieberle, P. (2013). Characterization of the Key Aroma Compounds in Two Bavarian Wheat Beers by Means of the

- Sensomics Approach. *Journal of Agricultural and Food Chemistry*, 61 (47), 11303–11311. <https://doi.org/10.1021/jf403912j>
- Li, N., Wang, Y., You, C., Ren, J., Chen, W., Zheng, H. & Liu, Z. (2018). Variation in Raw Milk Microbiota Throughout 12 Months and the Impact of Weather Conditions. *Scientific Reports*, 8 (1), 2371. <https://doi.org/10.1038/s41598018-20862-8>
- Liu ChunFeng, L.C., Dong JianJun, D.J., Zheng FeiYun, Z.F., Li YongXian, L.Y., Li Qi, L.Q. & Gu GuoXian, G.G. (2008). Application of fuzzy comprehensive evaluation in beer harmony characteristic tasting.
- Maicas, S. (2020). The Role of Yeasts in Fermentation Processes. *Microorganisms*, 8 (8), 1142. <https://doi.org/10.3390/microorganisms8081142>
- Matsushita, K., Toyama, H., Tonouchi, N. & Okamoto-Kainuma, A. (red.) (2016). *Acetic Acid Bacteria*. Springer Japan. <https://doi.org/10.1007/978-4-43155933-7>
- Meilgaard, M.C. (1982). Prediction of flavor differences between beers from their chemical composition. *Journal of Agricultural and Food Chemistry*, 30 (6), 1009–1017. <https://doi.org/10.1021/jf00114a002>
- Minervini, F., Lattanzi, A., De Angelis, M., Celano, G. & Gobbetti, M. (2015). House microbiotas as sources of lactic acid bacteria and yeasts in traditional Italian sourdoughs. *Food Microbiology*, 52, 66–76. <https://doi.org/10.1016/j.fm.2015.06.009>
- Nigam, P.S. & Singh, A. (2014). FERMENTATION (INDUSTRIAL) | Production of Oils and Fatty Acids. I: *Encyclopedia of Food Microbiology*. Elsevier. 792–803. <https://doi.org/10.1016/B978-0-12-384730-0.00112-9>
- Olaniran, A.O., Hiralal, L., Mokoena, M.P. & Pillay, B. (2017). Flavour-active volatile compounds in beer: production, regulation and control: Flavouractive volatile compounds in beer: production, regulation and control. *Journal of the Institute of Brewing*, 123 (1), 13–23. <https://doi.org/10.1002/jib.389>
- Parapouli, M., Vasileiadis, A., Afendra, A.-S. & Hatziloukas, E. (2020). *Saccharomyces cerevisiae* and its industrial applications. *AIMS microbiology*, 6 (1), 1–31. <https://doi.org/10.3934/microbiol.2020001>
- Peyer, L. (2017). Lactic acid bacteria fermentation of wort as a tool to add functionality in malting, brewing and novel beverages. <https://cora.ucc.ie/handle/10468/4694> [2024-05-15]
- Peyer, L.C., Zarnkow, M., Jacob, F., De Schutter, D.P. & Arendt, E.K. (2017). Sour Brewing: Impact of *Lactobacillus Amylovorus* FST2.11 on Technological and Quality Attributes of Acid Beers. *Journal of the American Society of Brewing Chemists*, 75 (3), 207–216. <https://doi.org/10.1094/ASBCJ-20173861-01>
- Pires, E.J., Teixeira, J.A., Brányik, T. & Vicente, A.A. (2014). Yeast: the soul of beer's aroma—a review of flavour-active esters and higher alcohols produced by the brewing yeast. *Applied Microbiology and Biotechnology*, 98 (5), 1937–1949. <https://doi.org/10.1007/s00253-013-5470-0>
- Ploier, B., Korber, M., Schmidt, C., Koch, B., Leitner, E. & Daum, G. (2015). Regulatory link between steryl ester formation and hydrolysis in the yeast *Saccharomyces cerevisiae*. *Biochimica et Biophysica Acta (BBA)-*

- Molecular and Cell Biology of Lipids*, 1851 (7), 977–986
- Preedy, V.R. (2011). *Beer in Health and Disease Prevention*. Academic Press.
- Quigley, L., O’Sullivan, O., Beresford, T.P., Ross, R.P., Fitzgerald, G.F. & Cotter, P.D. (2011). Molecular approaches to analysing the microbial composition of raw milk and raw milk cheese. *International Journal of Food Microbiology*, 150 (2–3), 81–94. <https://doi.org/10.1016/j.ijfoodmicro.2011.08.001>
- Quince, C., Walker, A.W., Simpson, J.T., Loman, N.J. & Segata, N. (2017). Shotgun metagenomics, from sampling to analysis. *Nature biotechnology*, 35 (9), 833–844
- Ripari, V., Gänzle, M.G. & Berardi, E. (2016). Evolution of sourdough microbiota in spontaneous sourdoughs started with different plant materials. *International Journal of Food Microbiology*, 232, 35–42. <https://doi.org/10.1016/j.ijfoodmicro.2016.05.025>
- Rodriguez, R.J., Low, C., Bottema, C.D. & Parks, L.W. (1985). Multiple functions for sterols in *Saccharomyces cerevisiae*. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism*, 837 (3), 336–343
- Ruiz, B., Chávez, A., Forero, A., García-Huante, Y., Romero, A., Sánchez, M., Rocha, D., Sánchez, B., Rodríguez-Sanoja, R. & Sánchez, S. (2010). Production of microbial secondary metabolites: regulation by the carbon source. *Critical reviews in microbiology*, 36 (2), 146–167
- Sahu, L. & Panda, S.K. (2021). Chapter 14 - Kefir, kombucha, and sour beers. I: Panda, S.K., Kellershohn, J., & Russell, I. (red.) *Probiotic Beverages*. Academic Press. 287–307. <https://doi.org/10.1016/B978-0-12-818588-9.00008-5>
- Şanlıer, N., Gökçen, B.B. & Sezgin, A.C. (2019). Health benefits of fermented foods. *Critical Reviews in Food Science and Nutrition*, 59 (3), 506–527. <https://doi.org/10.1080/10408398.2017.1383355>
- Sathiamoorthy, S., Malott, R.J., Gisonni-Lex, L. & Ng, S.H.S. (2018). Selection and evaluation of an efficient method for the recovery of viral nucleic acids from complex biologicals. *npj Vaccines*, 3 (1), 1–6. <https://doi.org/10.1038/s41541-018-0067-3>
- Shin, K.-S. & Lee, J.-H. (2019). Acetaldehyde contents and quality characteristics of commercial alcoholic beverages. *Food Science and Biotechnology*, 28 (4), 1027–1036. <https://doi.org/10.1007/s10068-019-00564-1>
- Smith, B.D. & Divol, B. (2016). *Brettanomyces bruxellensis*, a survivalist prepared for the wine apocalypse and other beverages. *Food Microbiology*, 59, 161–175. <https://doi.org/10.1016/j.fm.2016.06.008>
- Snyder, L.R., Kirkland, J.J. & Dolan, J.W. (2011). *Introduction to modern liquid chromatography*. John Wiley & Sons.
- Song, H. & Liu, J. (2018). GC-O-MS technique and its applications in food flavor analysis. *Food Research International*, 114, 187–198
- Spitaels, F., Van Kerrebroeck, S., Wieme, A.D., Snauwaert, I., Aerts, M., Van Landschoot, A., De Vuyst, L. & Vandamme, P. (2015a). Microbiota and metabolites of aged bottled gueuze beers converge to the same composition. *Food Microbiology*, 47, 1–11. <https://doi.org/10.1016/j.fm.2014.10.004>
- Spitaels, F., Wieme, A.D., Janssens, M., Aerts, M., Daniel, H.-M., Van Landschoot, A., De Vuyst, L. & Vandamme, P. (2014). The Microbial Diversity of Traditional Spontaneously Fermented Lambic Beer. Liles, M.R. (red.) (Liles, M. R., red.) *PLoS ONE*, 9 (4), e95384.

- <https://doi.org/10.1371/journal.pone.0095384>
- Spitaels, F., Wieme, A.D., Janssens, M., Aerts, M., Van Landschoot, A., De Vuyst, L. & Vandamme, P. (2015b). The microbial diversity of an industrially produced lambic beer shares members of a traditionally produced one and reveals a core microbiota for lambic beer fermentation. *Food Microbiology*, 49, 23–32. <https://doi.org/10.1016/j.fm.2015.01.008>
- Steensels, J., Daenen, L., Malcorps, P., Derdelinckx, G., Verachtert, H. & Verstrepen, K.J. (2015). Brettanomyces yeasts—From spoilage organisms to valuable contributors to industrial fermentations. *International journal of food microbiology*, 206, 24–38
- Stewart, G.G. (2015). Yeast quality assessment, management and culture maintenance1. I: *Brewing Microbiology*. Elsevier. 11–29. <https://doi.org/10.1016/B978-1-78242-331-7.00002-2>
- Styger, G., Prior, B. & Bauer, F.F. (2011). *Wine flavor and aroma. Microbiol Biotechnol* 38: 1145–1159.
- Suzuki, K. (2015). *Brewing microbiology managing microbes, ensuring quality and valorising waste*. Woodhead publishing series in food science, technology, and nutrition.
- Svedlund, N., Evering, S., Gibson, B. & Krogerus, K. (2022). Fruits of their labour: biotransformation reactions of yeasts during brewery fermentation. *Applied Microbiology and Biotechnology*, 106 (13), 4929–4944. <https://doi.org/10.1007/s00253-022-12068-w>
- Tan, Y., Du, H., Zhang, H., Fang, C., Jin, G., Chen, S., Wu, Q., Zhang, Y., Zhang, M. & Xu, Y. (2022). Geographically Associated Fungus-Bacterium Interactions Contribute to the Formation of Geography-Dependent Flavor during High-Complexity Spontaneous Fermentation. *Microbiology Spectrum*, 10 (5), e01844-22. <https://doi.org/10.1128/spectrum.01844-22>
- Tonsmeire, M. & Cilurzo, V. (2014). COMMERCIAL METHODS OF SOURING. I: *American Sour Beer*. Brewers Publications.
- Tran, A., Alby, K., Kerr, A., Jones, M. & Gilligan, P.H. (2015). Cost savings realized by implementation of routine microbiological identification by matrix-assisted laser desorption ionization–time of flight mass spectrometry. *Journal of Clinical Microbiology*, 53 (8), 2473–2479
- Van Leeuwen, C., Barbe, J.-C., Darriet, P., Geffroy, O., Gomès, E., Guillaumie, S., Helwi, P., Laboyrie, J., Lytra, G., Le Menn, N., Marchand, S., Picard, M., Pons, A., Schüttler, A. & Thibon, C. (2020). Recent advancements in understanding the terroir effect on aromas in grapes and wines: This article is published in cooperation with the XIIIth International Terroir Congress November 17-18 2020, Adelaide, Australia. Guests editors: Cassandra Collins and Roberta De Bei. *OENO One*, 54 (4). <https://doi.org/10.20870/oeno-one.2020.54.4.3983>
- Van Oevelen, D., Spaepen, M., Timmermans, P. & Verachtert, H. (1977). Microbiological aspects of spontaneous wort fermentation in the production of lambic and gueuze. *Journal of the Institute of Brewing*, 83 (6), 356–360
- Verachtert, H. & Iserentant, D. (1995). Properties of Belgian acid beers and their microflora. The production of Gueuze and related refreshing acid beers. *Cerevisia. Belgian Journal of Brewing and Biotechnology (Belgium)*, 20 (1)
- Wagg, C., Schlaeppli, K., Banerjee, S., Kuramae, E.E. & Van Der Heijden, M.G.A. (2019). Fungal-bacterial diversity and microbiome complexity predict ecosystem

- functioning. *Nature Communications*, 10 (1), 4841. <https://doi.org/10.1038/s41467-019-12798-y>
- Witrick, K., Pitts, E.R. & O’Keefe, S.F. (2020). Analysis of Lambic Beer Volatiles during Aging Using Gas Chromatography–Mass Spectrometry (GCMS) and Gas Chromatography–Olfactometry (GCO). *Beverages*, 6 (2), 31. <https://doi.org/10.3390/beverages6020031>
- Zaky, A., Glastras, S.J., Wong, M.Y.W., Pollock, C.A. & Saad, S. (2021). The Role of the Gut Microbiome in Diabetes and Obesity-Related Kidney Disease. *International Journal of Molecular Sciences*, 22 (17), 9641. <https://doi.org/10.3390/ijms22179641>
- Zheng, Y., Chang, Y., Xie, S., Song, J. & Wang, M. (2018). Impacts of bioprocess engineering on product formation by *Acetobacter pasteurianus*. *Applied Microbiology and Biotechnology*, 102 (6), 2535–2541. <https://doi.org/10.1007/s00253-018-8819-6>

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