MICROBIOLOGICAL PHASES OF SPONTANEOUSLY FERMENTED BEER

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ABSTRACT

Fermentation is traditionally divided into two types of fermentation bottom and top fermentation. There is a third type of fermentation, which is traditionally used for spontaneously fermented beer. Spontaneous fermentation runs slowly because starts without inoculation. All of the 100 species of yeasts and more than 50 species of bacteria that were obtained from this beer come from the environment. A wide spectrum of microorganisms is discriminated on base of substrate usability, pH and alcohol tolerance, temperature, and speed of growth. So, fermentation is divided into different phases as normal one-organism fermentation. Phases are: the phase of Enterobacteriaceae, the phase of main fermentation, the phase of acidification, and the phase of maturation. Every phase is specific for the mostly obtained microorganism, specific metabolites, or technological processes. During 3 years of fermentation in every case, a unique product with terroir will arise. Understanding this process is necessary to apply its interesting parts to new beverages productions and other fermentation technologies.

Keywords: spontaneous fermentation, beer, lambic, microbiological phases

INTRODUCTION

Throughout history, not only technology of multi-preparing and beer brewing were evaluated. For thousands of years, beer was fermented only with microorganisms from the environment. During those times, fermentation was unpredictable, which preached big failures and losses (Hornsey, 2003). The first pure culture was used in 1883 by Emil Ch. Hansen in Carlsberg brewery labs. This signified new age of brewing, with pure cultures, but without complex aromas and tastes (Hansen, 1883). This new technic exploded around Europe and the increase in lager brewing was significant (Libkind et al., 2011) and nowadays highest part of beer production is using Saccharomyces bayanus or Saccharomyces pastorianus (Bokulich and Bamforth, 2013). But in one region of only roughly 500 square-kilometers area around Brussel and Payottenland in the valley of Senna River, the knowledge of the oldest beer style survived. The terroir of this region provided a fully complex microbiota, which was necessary to produce high quality and constant products, so brewers were not confronted with necessary in the changing brewing process here (De Keersmaeker, 1996). Like in most countries, in Belgium the highest part of production is brewing in large brewing companies, too. Only 2.5 % of Belgium’s beer production are Lambic beer, for 20 century the number of small or family-operated brewery shrunk from 3000 to 60. These days about 20 breweries still produce Lambic beer. Some of the breweries were reclassified from breweries to blenders, which are not brewing beer but buying a wort and only fermenting it as a blend of their own product (Guinand, 1990).

SPONTANEOUS FERMENTATION

Fermentation has been running for 3 years (De Keersmaeker, 1996). The principle of spontaneous fermentation is not picking pure yeast culture into the cold wort. Some breweries are using a system of back-slopping, where part of an old batch is added into a new batch, normally it is the bottom of fermented volume, where is a higher concentration of flocculated yeast. Another technique where some part of post-main fermentation species from cask are added into beer (fermented with pure culture) is used too. Any of these techniques are not connected with spontaneous fermentation and this beer will not be possible to call spontaneously fermented beer. But unfortunately, it is used. Spontaneous fermentation must ferment without any targeted inoculation. The main part of the microbiota is obtained during the cooling of wort (Bamforth, 2005; Spietaels et al., 2014; Van Oevelen et al., 1977). New studies show that the microbiota of air is so cool to cool the wort at 20 °C for one night because only natural cooling is used. Coolers are constructed in the attic traditionally, cooling running with a low level of wort 10-20 cm and windows in the attic are opened. After cooling wort is transported to old wine wooden casks of red wine and fermented in one step without another transport (like is normal in nowadays brewing systems of CK tanks) for 3 years. The Casks are stored in the cellar where the ambient temperature culminates between 15-25 °C. There are not any coolers or heaters, during summer temperature can attack 25 or more degrees, and during winter temperature shrinks. The studies looked at microbiota and metabolites production and divided fermentation into four phases: Phase of Enterobacteriaceae, phase of the main fermentation, phase of acidification, and phase of maturation (Esslinger, 2009; Spietaels et al., 2014; Van Oevelen et al., 1977).

PHASE OF ENTEROBACTERIACEAE

In older studies the Enterobacteriaceae phase was described as about 1-month length (Martens et al., 1991), we can say that methods at that time were constructed on different bases. Nowadays studies talk about a shorter phase of about one week of fermentation (De Roos et al., 2018; De Roos and De Vuyt, 2018). Enterobacteriaceae are facultative anaerobic bacteria that are using Embden-Meyerhof Pathway to metabolize monoasacharides and carry out mixed-acid fermentation which produces lactic acid, acetic acid, succinic acid, and formic acid. Most influences are lactic and acetic acid because of their impact on taste and pH level (Van Vuuren and Priest, 2003). There was obtained different species of Enterobacteriaceae in lambic beers: Klebsiella pneumonia, Enterobacter cloacae, Hafnia protea, Hafnia alvei, Citrobacter freundii, Serratia strains, and Proteus mirabilis. In the past Enterobacteriaceae family was neglected without impact on the fermentation process, during times with new methods like chromatography their impact was proven. They can produce off-flavor metabolites like sulfur compounds mainly dimethyl sulfide. Generously, their occurrence relates to phenolic and medical off-flavors in beer and indirectly with diacetyl production. Van Vuuren's team firstly find Enterobacter agglomeraes (Martens et al., 1991; Van Vuuren et al., 1979; Vriesekoop et al., 2013). Martens et al. (1991) found the top of Enterobacteriaceae in eight days of fermentation, after this (10⁶ CFU/mL was detected density in top) population of bacteria slowly shrunk and after 30 – 40 days no one of Enterobacteriaceae was important to protect Lambic production. Air from modern urbanized Brussels is poor to microbiota. Studies talk about low or lost inoculum during the cooling of beer in modern industrial Lambic production and the biggest part of contamination is obtained from casks, which are not sterilized like in pure culture fermented breweries, but only cleaned with water (De Roos et al., 2019; Spietaels et al., 2014; Spietaels et al., 2015). Wort must be brewing in cold months when the temperature of the air is so cool to cool the wort at 20 °C for one night because only natural cooling is used. Coolers are constructed in the attic traditionally, cooling running with a low level of wort 10-20 cm and windows in the attic are opened. After cooling wort is transported to old wine wooden casks of red wine and fermented in one step without another transport (like is normal in nowadays brewing systems of CK tanks) for 3 years. The Casks are stored in the cellar where the ambient temperature culminates between 15-25 °C. There are not any coolers or heaters, during summer temperature can attack 25 or more degrees, and during winter temperature shrinks. The studies looked at microbiota and metabolites production and divided fermentation into four phases: Phase of Enterobacteriaceae, phase of the main fermentation, phase of acidification, and phase of maturation (Esslinger, 2009; Spietaels et al., 2014; Van Oevelen et al., 1977).
detected. In a new study (De Roos and De Vuyst, 2019) phase of fast-growing and top very was similar, but the phase ended under 2 weeks of fermentation. Fermentation was faster and the equal pH value after 2 weeks was under 4. Van Vuuren et al. (1979) wrote that Enterobacteriaceae are sensitive to pH under 5.5 and alcohol under 2%. After the first week, the researchers detected different microorganisms in two studies, but in conclusion, we can say, that in both experiments the phase end after the pH value falls under 4, after consumption of all glucose and fructose like in De Roos et al. (2018a) and when alcohol concentration exceeds 2% this phase end. In old and nowadays studies we can see differences, but first what we need to see is how easy fermentation because government authorities and European Union made press on breweries to reduce the occurrence indication of fecal contamination during processing. Unfortunately, Hanseniaspora uvarum was not detected in beer produced with lactic acid addition. This specie relates to low fermentative capacity but is commonly found during the spontaneous fermentation of wines, and cider, where contribution to flavor complexity is increasingly appreciated (Bezerra-Bussoli et al., 2013; Spitaels et al., 2015).

Acetic acid bacteria are obtained here too, mostly Acetobacter and Gluconobacter species. In the first part of the fermentation, Acetobacter orientalis is the predominant acetic acid bacteria. The spatial analysis shows significant diversity between the concentration of acetic acid during the first phase. The acetic acid was produced in the top part of the beer, where is beer in contact with air. So, more oxygen starts higher acetic acid production in this part (De Roos et al., 2018b). Meanwhile in American coolship Ale most detected bacteria Klebsiella oxytoca and Enterobacter agglomerans but also Enterobacter ludwigi, Enterobacter cloacae, Enterobacter mori, Klebsiella pneumonia, and Serratia urethritis. Yeasts are Candida krusei, Pichia fermentans/kluyveri, Cryptococcus keutzingii a Rhodotorula mucilaginosa and predominant Rhodotorula mucilaginosa which after the first week of fermentation occupied 40% of obtained strains, so terror of the different continent has an impact on microbiota during this phase. In American coolship Ale, some Enterobacteriaceae were detected over the first 12 weeks, but molecular methods detect them sometimes later, this is big different in opposite to modern Lambic production, where lactic acid is added and detection of Enterobacteriaceae stop after 40 days of fermentation (Bokulich et al., 2012).

PHASE OF MAIN FERMENTATION

Saccharomyces follows the decline of Enterobacteriaceae, and they start growing, possibly it is related to relief from competition and acclimation to metabolites of Enterobacteriaceae and oxidative yeasts like carboxylic acids. Fermentation run from 2. to 9. week very quickly, after this time 80% of the extract is consumed during summer when the temperature is too high to detect them sometimes later, this is big different in opposite to modern Lambic fermentation because LAB start growing and are connected with this phase are

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PHASE OF MATURATION

Maturity is the last phase of beer production, in new studies, authors did not see any big differences between the phase of acclimation and the phase of maturation (Bokulich et al., 2012; De Roos et al., 2018a; Spitaels et al., 2014; Spitaels et al., 2015) but this is only microbial aspect. Technological aspects are different, Lambic beer ferment in open fermenters, so beer has a low CO2 level and is sensory dull. For carbonization, two ways are used. The first way is mixing traditional LAB start growing and are connected with this phase are

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used for fermentation. In years with a good harvest, big portions of fruit were added to beer. Fruit is rich not only in sugar but organic acids too. Analogous to other lactic acid bacteria in wine production, _Pediococcus damnosus_ can perform a malolactic fermentation during fruit fermentation in spontaneous fermentation. This process grades the malic acid from fruit (apples or grapes) and changes it to lactic acid which is sweeter and softer (Versari et al., 1999; Zhang and Lovitt, 2006). Fermentation is connected with Brettanomyces and _Pediococcus_ mostly. The concentration of 4-ethylphenol and 4-ethyl guaiacol grow as well as lactic acid and ethyl lactate. Degradation of isomaltose accentuates continued. Gueuze beer is stable for 10 years (Spitaels et al., 2015).

**CONCLUSION**

The traditional way of beer fermentation is a complicated and not always successful process of production, big losses, and inconsistent products are the biggest challenges here, but the final product is unique and highly valuable. Nowadays, when we understand mechanisms in the processing, we can apply parts of these techniques or use specific microorganisms which are responsible for accepting metabolites. Part of wild yeast obtained from spontaneous fermentation has a small fermentation capacity, and is possible to use for low-alcohol or non-alcoholic beer production. Brettanomyces species live in renaissance nowadays, new techniques and knowledge of their behavior enable them to produce different beverages with a new dimension of taste and flavors. B-glucosidase was isolated and now is using the food industry, this is only one of the potential applications of spontaneous fermentation in the modern food industry.

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