ESTERS AND HIGHER ALCOHOLS PRODUCTION ASSESSED IN LAGER BEER DURING PRIMARY FERMENTATION PERFORMED AT INDUSTRIAL SCALE

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Abstract
Secondary metabolites such as esters and higher alcohols produced by yeasts during fermentation were reported to have large effects on the final sensorial quality of fermented beverages. This work aimed to assess the fluctuations in beer esters and higher alcohols content due to process parameters variation during primary fermentation. Therefore, fermentation trails were conducted at industrial scale using a commercial lager brewing yeast. Esters and higher alcohols profiles were determined following fermentation efficiency by monitoring yeast growth, ethanol synthesis, specific gravity, pH and tank pression throughout the fermentative process. Results showed increase in yeast cells (58 ± 6,55x10⁶ cells/ml) due to the high yeast multiplication resulting in intense metabolic activity. Indeed, increased ethanol yield (3,83 ±1,07 %) recorded after 5 days demonstrated higher yeast activity during primary fermentation. Yeast grow was probably enhanced by wort fermentable sugars and free amino acid content. Interestingly, the production of 2-Methyl-1-butanol (44,82 ± 4,12 mg/l), and ethyl acetate (14,89±4,58mg/l) was significantly improved during the primary fermentation. However, acetaldehyde content increased simultaneously only within 3 fermentation days (10,51 ± 0,012mg/l). These flavor-active compounds may contribute positively to the overall sensorial properties of the final beer.

Keywords: sensory properties, fermentation, esters, higher alcohols, yeast.

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1. INTRODUCTION

Brewing yeasts were reported to produce secondary metabolites such as flavor-active substances including organic acids, higher alcohols, volatile esters, phenolic compounds, etc. in alcoholic beverages (Verstrepen et al., 2003; Aritomi et al., 2004). Among secondary metabolites produced by yeasts volatile esters are known as a vital group of aromatic compounds conferring the fruity character to alcoholic beverages (Liu and Quek, 2016; van Rijswijck et al., 2017). The most important flavor-active esters in beer are ethyl acetate (solventlike aroma), isoamyl acetate (fruity, banana aroma), ethyl caproate and ethyl caprylate (sour apple), and phenyl ethyl acetate (flowery, roses, honey) (Saerens et al., 2008). It is well established that the flavor attributes of beer are critical to its overall acceptance by consumers.

However, many parameters are known to affect ester production in beer, including brewing yeast strain, wort composition, high-gravity brewing (Lei et al., 2012), wort oxygenation (Thurston, 1980), unsaturated fatty acid levels of the wort (Marchesini and Poirier, 2003), hydrostatic pressure of the fermentation tank (Landaud et al., 2001), and fermentation temperature (Suomalainen, 1981). Therefore, monitoring flavor-active esters formation in order to be able to better control their levels in the end product is of major industrial interest (Dufour et al., 2002, Carrau et al., 2008). Currently, studies are focused on optimization of technical parameters in the fermentation process in order to control the flavor of beer. It has been reported that current challenge in brewing industries is how to apply the findings of laboratory and pilot-plant fermentations to industrial process. Hence, understanding metabolites production in beer via physiological behavior of yeast to better control their levels in the end product is of major industrial interest. In this study, yeast physiological fluctuations due to process

parameters variation during primary fermentation are supposed inducing outstandingly fluctuations in beer esters and higher alcohols content. Therefore, fermentation trails were conducted at industrial scale using a commercial lager brewing yeast. Esters and higher alcohols profiles were determined at different period following fermentation performance by monitoring yeast growth, ethanol synthesis, specific gravity, pH and tank pressure throughout the fermentative process.

2. MATERIALS AND METHODS

Yeast

A lager brewing strain of *Saccharomyces cerevisiae* var. uvarum (Unicer) was used in our work. Lager fermentation trials were conducted in duplicate at industrial scale in 1000-HL horizontal fermenters (Horaps).

Wort preparation and fermentation conditions

The wort (14 °P) used for trials was brewed according to standard production procedures. It was filtered and cooled before fermentation. Wort samples were aseptically dispensed into vessels (250 ml) at days 1, 2, 3, 4 and 5 to be analyzed. Lager wort used in fermentation process was prepared using canned-hopped malt extract according to the manufacturer's instructions.

Primary fermentation was set-up to determine esters and higher alcohols in beer. In order to preserve the fermentation conditions as similar as possible, the same brew was equally divided for fermentations 1, 2, 3 and 4. Approximately 1000 HL of the wort was transferred into each of the four horizontal fermenters (horap) and aerated in-line. Fermentations were conducted using a bottom fermenting yeast (Guido et al., 2004). The fermentation operating parameters are outlined in Table 1.

Cell count

Yeast cell counts were performed using a NucleoCounter YC-100. The apparatus consists of a camera and an integrated fluorescence microscope designed to detect signals from the fluorescent dye, propidium iodide (PI) bound to yeast DNA.

Ethanol, esters and higher alcohols analysis

Ethanol, esters and higher alcohols were analyzed by chromatographic method (static headspace) using a GC Peken Elmer gas chromatograph equipped with a flame ionization detector (FID) and a CombiPAL Autosampler as recommended by the European Brewery Convention (Analytica EBC, Neurnberg: Fachverlag Hans Carl, 2000). Solutions containing propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl acetate, 3-methyl butyl acetate, 2-methylbutylacetate, ethyl caproate, butyric acid ethyl ester and isobutylacetate were prepared and used as standards.

Table 1: Process parameters values recorded during primary fermentation in horizontal fermenters. Data are expressed as means± standard deviation (n=3).

Fermentation parameters	pH	Temperature (°C)	Pressure (Bar)
Day 1	4,75 ± 0,45	10,5 ± 0,99	0,017 ± 0,018
Day 2	4,6 ± 0,04	10,5 ± 0,36	0,0025 ± 0,002
Day 3	4,58 ± 0,13	10,3 ± 0,17	0,005 ± 0,004
Day 4	4,45 ± 0,07	10,4 ± 0,04	0,0075 ± 0,01
Day 5	4,4 ± 0,07	10,3 ± 0,1	0,005 ± 0,004

3. RESULTS AND DISCUSSION

Specific gravity, ethanol and yeast count assessment

During fermentation process in brewery the yeast must adapt to constantly changing environmental conditions including fluctuations of carbon and nitrogen sources, temperature oxygenation, and stresses (hyperosmotic stress, high ethanol concentration, anaerobiosis). Yeast cells adapt their physiology to environmental changes by reorganizing their genomic expression and so changing the patterns of cellular proteins and metabolites (Rautio et al., 2007).

Curves demonstrating variations of specific gravity, ethanol production, and yeast count during five days of fermentation in horizontal fermenters are shown in Figure 1.

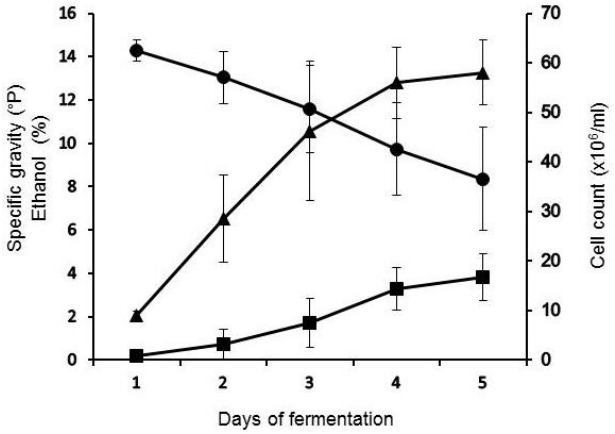

Figure 1: Curves showing variation of (●) specific gravity, (■) ethanol production, and (▲) yeast count during five days of fermentation in horizontal fermenters. Data are expressed as means± standard deviation (n=3).

Growth curve (Figure 1) showed an important increase in yeast cells ($58x10^6$ cells/ml) due probably to high metabolic activity allowing intense yeast multiplication. On the other hand, increasing ethanol yields (3,83%) following specific gravity decrease (from 14,27 to 8,36 °P) recorded during 5 days of fermentation demonstrated higher fermentative capacity of brewing yeast. Additionally, the yeast showed ethanol tolerance based on cell count curve improving fermentation performance after 3 days of fermentation.

Several factors were reported to influence the yeast fermentative capacity, particularly the composition of wort depending on fermentable sugars (glucose, fructose, maltose and maltotriose), free amino acid content, lipid composition, wort oxygenation, and temperature (Guido et al., 2004; Hiralal et al. 2014). Indeed, Lei et al. (2012) demonstrated that both the wort gravity and nitrogen level have significant impacts on the growth rate, viability, flocculation, and gene expression of brewer's yeast and the levels of flavor volatiles.

Higher alcohols and esters production

It has been reported that physiological condition of yeast may influence the levels of organic acids, esters, higher alcohols, aldehydes and diacetyl throughout fermentation and maturation, and consequently contribute to the overall organoleptic properties of the final beer. Even though aroma-active substances are only produced in low concentration they can affect beer flavor notably by synergy effects. However, the production of these secondary metabolites depends on yeast viability demonstrating the ability of cells to grow, reproduce and interact with their immediate environment (Smart et al., 1999, Saerens et al., 2008). Fluctuations of higher alcohols and esters production assessed during primary fermentation in horizontal fermenters are displayed in Figures 2 and 3.

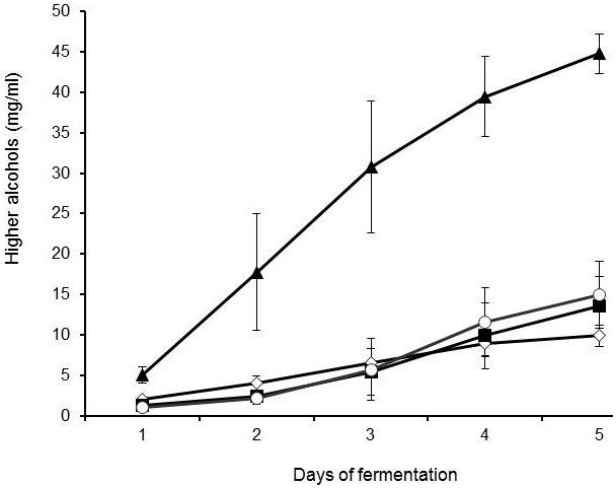

Figure 2: Higher alcohols content fluctuations assessed during primary fermentation in horizontal fermenters. (▲) 2-methyl-1 butanol, (○) 3-methyl-1 butanol, (■) 2-methyl-1 propanol, (◊) Propanol. Data are expressed as means± standard deviation (n=3).

Interestingly, several higher alcohols (Figure 2) including 2-methyl-1 butanol, 3-methyl-1 butanol, 2-methyl-1 propanol, and propanol were produced in beer at different level during primary fermentation. The most important higher alcohol produced was 2-methyl-1 butanol (44,82 mg/ml). It has been reported that some compounds such as Maillard reaction products formed during malt production can influence the synthesis of higher alcohols and esters in beer fermentations. For example, Dack et al. (2017) demonstrated that higher alcohol levels were significantly higher in dark malt fermentations, while the synthesis of esters was inhibited, due to possible suppression of enzyme activity and/or gene expression linked to ester synthesis.

However, esters production (Figure 3) was low compared to higher alcohols content in beer during primary fermentation. Among esters secreted by brewing yeast, ethyl acetate was the most dominant (14,89 mg/ml).

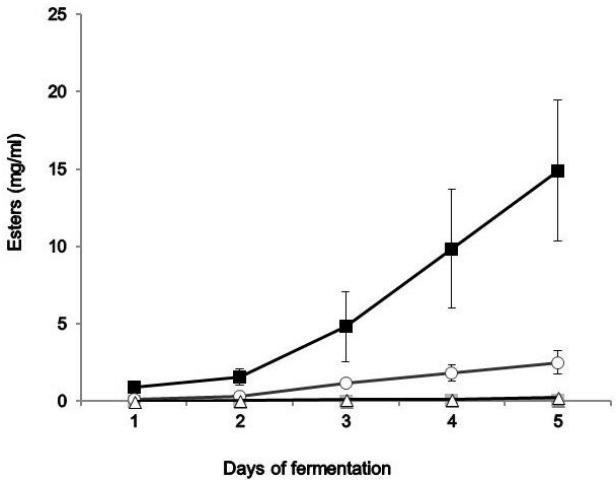

Figure 3: Beer esters content fluctuations assessed during primary fermentation in horizontal fermenters. (■) 2-methyl-proyl aceate, (○) Isoamyl acetate, (▲) Ethyl butyrate, (■) Ethyl acetate, (Δ) Ethyl caproate. Data are expressed as means± standard deviation (n=3).

Saerens et al. (2008) reported that acetate esters (acid group is acetate and alcohol group is ethanol or complex alcohol derived from amino acid metabolism) are produced at much higher levels and therefore are easier to measure compared to ethyl esters (alcohol group is ethanol and acid group is a medium-chain fatty acid). Unlike acetate ester excretion, which is rapid and complete, the transfer of ethyl esters to the fermenting medium decreases drastically with increasing chain length (Nykanen and Nykanen, 1977). Acetate ester production gradually increases with increasing fermentation temperature (Saerens et al., 2008). Ocvirk et al. (2018) reported that deviations in beer aroma are not a consequence of a permanent repeatable error in brewing process, but, they are a consequence of alcoholic fermentation. According to Nordström (1962), esters synthesis in yeast cells is catalyzed by ester synthase (acyl transferase). It has been reported that even small changes in the concentrations of these secondary metabolites can have large effects on the final sensorial quality of beer (Saerens et al., 2008).

According to literature, high wort gravity leads to an increased fermentation time, since it simply takes longer for the yeast to metabolize higher concentrations of fermentable sugars. Additionally, there are important interactions between process variables, i.e. temperature with initial wort gravity, and initial air saturation with wort gravity. Under conditions of higher gravity, ester synthesis is substantially promoted. At higher temperatures, ester yield increases at the expense of higher alcohol yield in equivalent operating conditions (Brown and Hammond, 2003). Additionally, wort oxygenation was shown to reduce ester production due to the decreased expression of the alcohol acetyl transferase gene ATF1 (Verbelen et al., 2009).

Our results are in accordance with those reported by Guido et al. (2004) demonstrating that fermentation efficiency and final product quality are intimately linked with the amount and vitality of the yeast being pitched during brewing process. Heggart et al. (2000) showed that yeast activity may influence not only esters and higher alcohols production, but also the levels of organic acids, aldehydes and diacetyl throughout fermentation and maturation. These metabolites were found to contribute to the overall organoleptic properties which are

important quality criterion for the final beer.
Hazelwood et al. (2006) demonstrated that *Saccharomyces cerevisiae* can use many amino acids, such as leucine, tyrosine, phenylalanine and methionine, through the Ehrlich pathway (catabolism) producing fusel acids and alcohols, along with their derived esters, are important contributors to beer and wine flavor.

4. CONCLUSION

High performance in primary fermentation increased higher alcohols and esters production in beer. However, the absolute amount of flavour-active compounds is not really relevant by itself for beer flavour; but their real impact should be checked through synergy with other flavour compounds present in the beer. Our results are evidence at industrial scale on the importance of the yeast physiological behavior for the production of beers with higher flavour active compounds. Further studies should be focused on beer sensorial proprieties to assess the effects of these compounds on organoleptic characteristics of end product.

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