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SURVIVAL OF SACCHAROMYCES CARLBERGENSIS ASSOCIATED TO DIACETYL EVOLUTION DURING WORT FERMENTATION

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ABSTRACT

In the present study, the different steps of brewing were described and yeast population as well as diacetyl production was analyzed during wort fermentation in order to improve the quality of final beer. Steps in the brewing process were composed of malting, milling, mashing, lautering, boiling, fermenting, conditioning, filtering, and packaging. Yeast grew according to a growth curve including a brief lag, exponential, stationary and declined phases. Diacetyl production was observed the eleventh day of fermentation at rate of 0.53 mg/L which decreased to reached 0.14 mg/L at fourteenth day. Young yeasts gave better multiplication and flocculation factors compared to the old one. Our study revealed that the time taken for diacetyl reduction to reach below 0.15 ppm varied from 3 to 4 days depending on yeast generation. This study gave valuable information for better understanding of the fermentation and diacetyl production which can be used to improve beer quality.

Key words: brewing, yeast, diacetyl, fermentation, multiplication and flocculating factors.

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1. INTRODUCTION

Brewing is an ancient art and is one of the oldest biotechnologies utilized by humans. It may be done in a brewery by a commercial brewer or at home by a variety of traditional methods such as communally by the peoples in Burkina Faso when making dolo. Economically, brewing is one of the most important biotechnological industries [1] and global beer production takes place on an unprecedented scale [2].

Steps in the brewing process include malting, milling, mashing, lautering, boiling, fermenting, conditioning, filtering, and packaging. After yeast is pitched into beer, the yeast undergo a lag phase, followed by an exponential phase during which yeast build amino acids, proteins, and other cell components[2]. One of the amino acids produced by yeast is valine. An intermediate compound in valine production is called acetolactate. Not all of the acetolactate produced eventually becomes valine; some will leak out of the cell and into the beer. This acetolactate is then chemically converted to diacetyl in the beer. Extensive research has been conducted on this compound in recent years. Factors that influence the diacetyl level in beer are fermentation temperature, aeration level, bacterial contamination and the yeast strain [2].

However the presence of diacetyl (2,3-butanedione), an important flavour active, oxidative compound in beer results in an unpleasant butterscotch-like flavor [3]. Furthermore, diacetyl negatively impacts cellular health and has been associated with neurodegenerative diseases and general cell aging amongst others.

The reduction of this compound is therefore essential for cellular health. Most research is oriented towards accurate diacetyl detection and quantification [4,5,6] and prediction [1].

The present study aims to describe the different steps of brewing and to analyze diacetyl production during beer production in order to improve the quality of final beer.

2. MATERIALS AND METHODS

2.1. Fermentations and Sample Collection

In the present study, brewing processing was followed and the sterile wort obtained was inoculated with yeast (*Saccharomyces carlbergensis*) at a pitching rate of 10^7 cells/ml in the tank. The wort was fermented at 20°C for fourteen (14) days and samples were collected daily for viable count (CFU/ml), pH, multiplication and flocculation factors, diacetyl quantification and time for diacetyl reduction during fermentation.

Cell count was done by mixing 1mL of fermenting wort with 3 mL of methylene blue and 2 mL of distilled water; a counting chamber (Thoma's hemocytometer) was used to determine cell count.

Multiplication factor (Fi) of yeast in samples was obtained by calculating the ratio between the highest number of yeasts during fermentation period and the initial number of yeasts in the inoculum.

Flocculation factor (Fi') of yeast in samples was defined as the ratio between the highest number of yeasts during fermentation period and yeast number at the end of fermentation.

The content of diacetyl in fermenting wort was assessed according to the modified method described by [7]: 5 tubes, each containing 5 mL of sample, were immersed in a 22-23°C water bath. 0.6 mL of α-

naphtol, 0.2 mL of creatin and 4.2 mL of distilled water were added successively to each tube after vortexing. The absorbance was then read at 540 nm.

The time taken for diacetyl reduction below 0.15 ppm was determined using four generations of yeast (G_0, G_1, G_2, G3 and G_4).

2.2. Statistical Analysis

The parameters evaluated in the present study were assessed in triplicate (n=3) and reported as average ± standard deviation.

3. RESULTS AND DISCUSSION

3.1. Brewing Process

The different steps in the brewing process are summarized in figure 1 and included malting, mashing, lautering, boiling, fermenting, conditioning, filtering, and packaging [8].

Malting is the process where barley grain is made ready for brewing. Malting is broken down in order to help to release the starches in the barley [8, 9].

Mashing converts the starches released during the malting stage into sugars that can be fermented. The milled grain is mixed with hot water in a large vessel known as a mash tun. In this vessel, the grain and water are mixed together to create a cereal mash. During the mash, naturally occurring enzymes present in the malt convert the starches (long chain carbohydrates) in the grain into smaller molecules or simple sugars (mono-, di-, and tri-saccharides). This "conversion" is called saccharification.

Lautering: the result of the mashing process is a sugar-rich liquid or "wort", which is then strained through the bottom of the mash tun in a process known as lautering. Prior to lautering, the mash temperature may be raised to about 75–78°C (known as a mashout) to free up more starch and reduce mash viscosity [10].

Boiling: the wort is moved into a large tank where it is boiled with hops and sometimes other ingredients such as herbs or sugars. This stage is where many chemical and technical reactions take place, and where important decisions about the flavour, colour and aroma of the beer are made. The boiling process serves to terminate enzymatic processes, precipitate proteins, isomerize hop resins, concentrate and sterilize the wort. Hops are added to give biological stability through their antiseptic value, to add flavor and aroma, and to improve the foaming properties and colloidal stability of the beer [11]. At the end of the boil, the hopped wort settles to clarify in a vessel called a "whirlpool", where the more solid particles in the wort are separated out. After the whirlpool, the wort is drawn away from the compacted hop trub, and rapidly cooled via a heat exchanger to a temperature where yeast can be added. It is very important to quickly cool the wort to a level where yeast can be added safely as yeast is unable to grow in very high temperatures and will start to die in temperatures above 60°C [12].

Fermentation of wort: After the wort goes through the heat exchanger, the cooled wort goes into a fermentation tank. A type of yeast is selected and added, or "pitched", to the fermentation tank. At this point the pH of the wort is generally between 5.3 and 5.5 [11].When the yeast is added to the wort, the fermenting process begins, where the sugars turn into alcohol, carbon dioxide and other components. When the fermentation is complete, the beer may be racked into a new tank, called a conditioning tank [8].

Conditioning, filtering and Packaging: Conditioning of the beer is the process in which the beer ages, the flavour becomes smoother and flavours that are unwanted dissipate [13]. After conditioning for a week to several months, the beer may be filtered and force carbonated for bottling or fined in the cask [14].

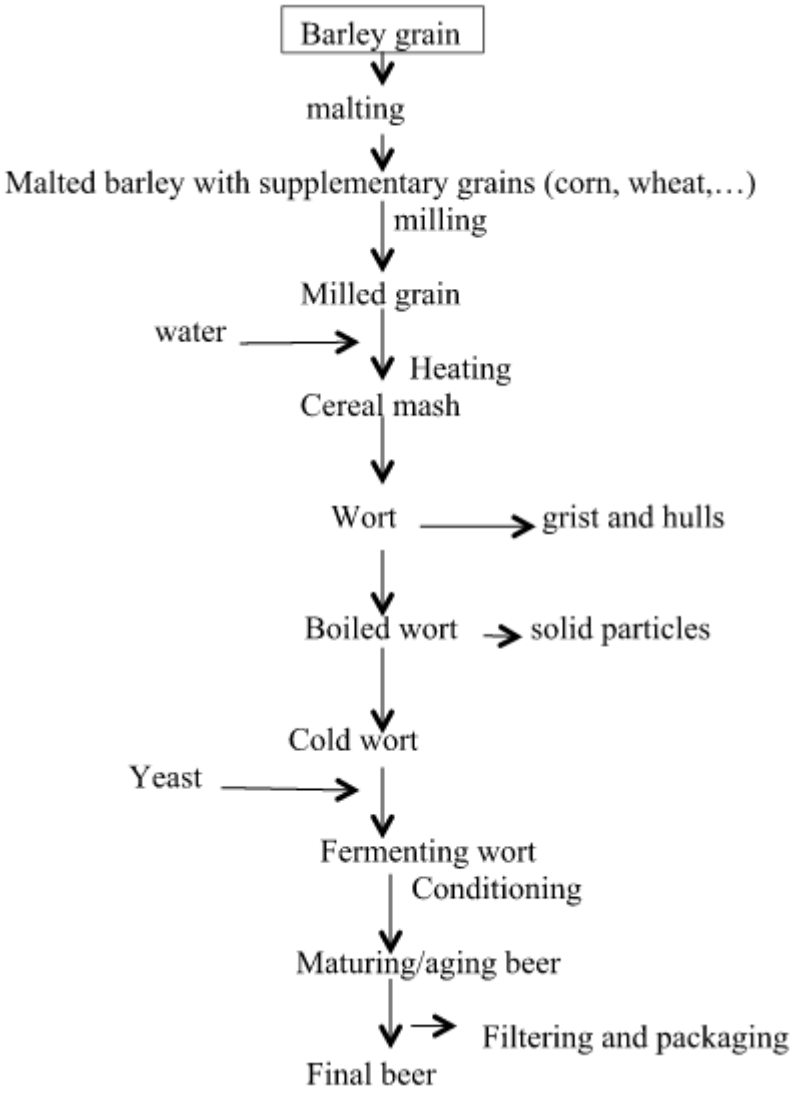

Figure 1 The different steps of brewing process

3.2. Evolution of Yeast Population and Diacetyl during Fermentation

As seen in figure 2, the yeasts in the wort had approximately 7.21 log (CFU/ml) at inoculation (t_0) and grew to 7.81 log (CFU/ml) by the seventh day. The population of yeasts was 7.36 log (CFU/ml) at the fourteenth day. The pH decreased from 5.6 at the fermentation beginning to 4.01 at fermentation end.

Yeast grew according to a growth curve which means that the growth behavior changes with time due to depletion of nutrients. In the beginning, the yeast undergoes a brief lag phase. During the lag phase yeast gets prepared for growth by synthesizing essential constituents. After the lag phase, the yeast enters the exponential phase where growth takes place. Yeast growth continues until nutrients are depleted and a phase called stationary phase is reached where the net growth is zero [15].

The pitching rate in our study is similar to those recommended by [16]. The pitching rate for fermentations is important because if the pitching rate is too low, the fermentation will take too long time resulting in an inefficient process. If the pitching rate is too high, it can lead to decrease viability of yeast which is to be collected and repitched from the fermentation. In addition, loss of bitterness and filtration problems can result from over-pitching [17].

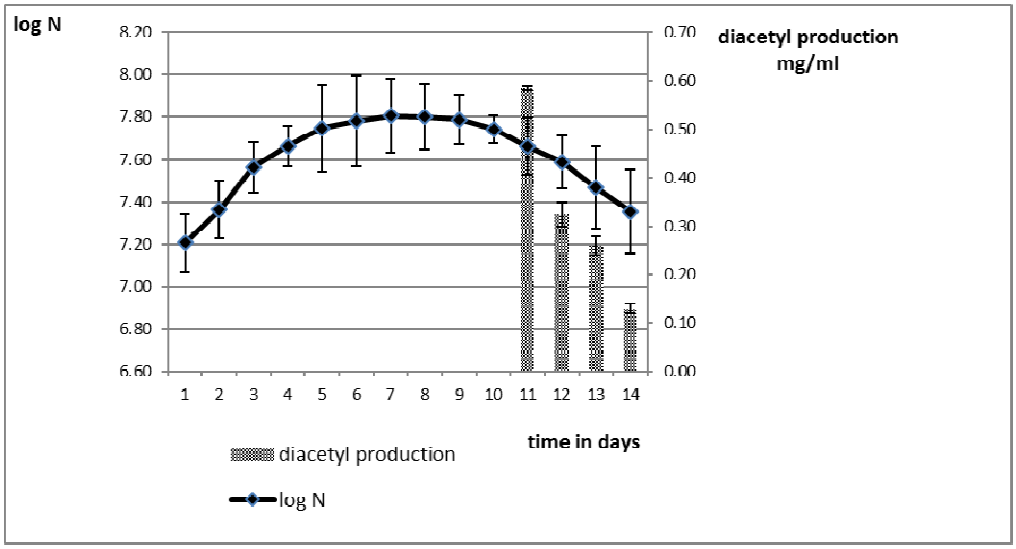

Figure 2 yeast population and diacetyl produced by *S. carlbergensis* strains determined after incubation in wort for 14 days.

Diacetyl is formed from acetolactate in the fermenting beer outside the yeast cells. It is then removed by the yeast cells and enzymically reduced to acetoin and butanediol as presented in figure 3. Our study revealed that diacetyl production was not observed during the first ten days of fermentation (figure 2). Diacetyl production was observed the eleventh day of fermentation at rate 0.53 mg/L. This value decreased during the last fermenting time to reached 0.14 mg/L at fourteenth day corresponding to 26.42% of reduction. After two weeks of fermentation, the diacetyl concentration in the final beer did not disappear completely but was below the low threshold level of 0.15 ppm. Similar tendency was observed by [11] who found that cells of *Streptococcus diacetilactis* destroyed diacetyl from solutions at a rate almost equal to that achieved by the addition of live, whole yeast cells. [18] stated that the amount of diacetyl produced during fermentation can be affected by modifying process conditions, wort composition or fermentation technique, or by yeast strain development through genetic engineering or adaptive evolution. In addition they found *Aerobacter aerogenes* strains to have considerably diacetyl reductase activity in beer [11]. A survey conducted by [19] showed a diacetyl range of 0.40 to 0.96 ppm for six samples of Russian beer tested.

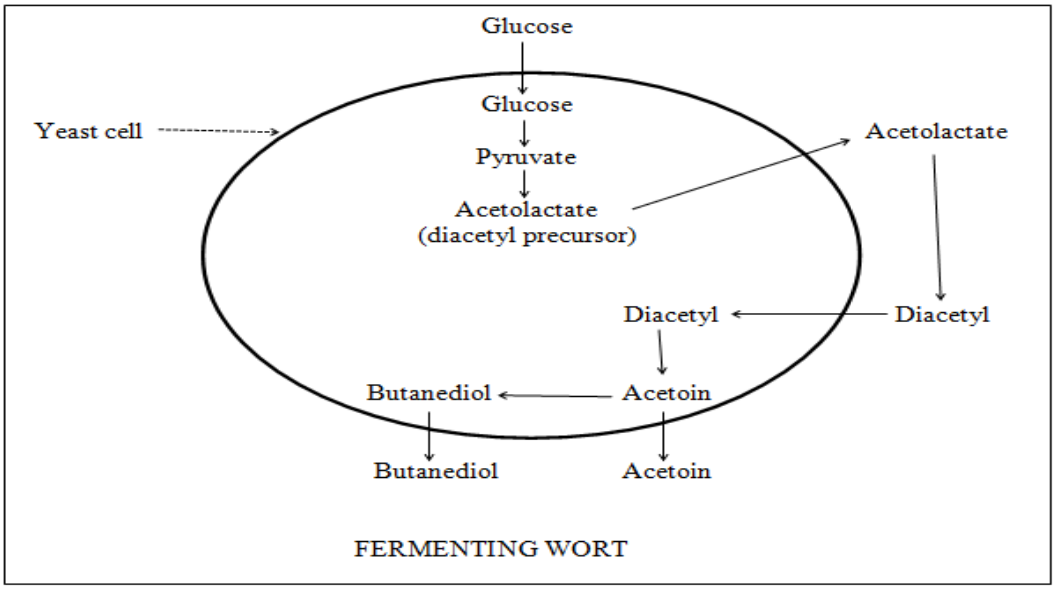

Figure 3 Compounds involved during diacetyl formation and reduction inside and outside of the yeast cell

The diacetyl must not be present at higher amounts than 0.15 ppm for desirable beer. Hence, time for diacetyl reduction below 0.15 ppm was determined using four generations of yeast (G_0, G_1, G_2, G3 and G_4). Our study revealed that the generation of yeast affects the time taken for diacetyl reduction to reach below 0.15 ppm. Results showed that the diacetyl was reduced to below 0.15 ppm after 4 days for initial generation of yeast (G_0). Three days were necessary for generations G_1 and G_2 to reduce diacetyl content to below 0.15 ppm. As for the 3rd and 4th generation of yeast, 3.5 days were necessary to reduce diacetyl content.

Multiplication factor (Fi) and Flocculation factor (Fi') were also studied using four generations of yeast (G_0, G_1, G_2, G3 and G_4) in fermenting wort. The results (Figure 4) showed the third generation of yeast (G3) to present the best multiplication (Fi=5.54) and flocculating (Fi'=3.29) factors. Whereas the initial cell (Go) showed lower multiplication (Fi=3.83) and flocculating (Fi'=2.09) factors. We can consider that young yeasts are efficient multiplication and flocculating property compared to the old one.

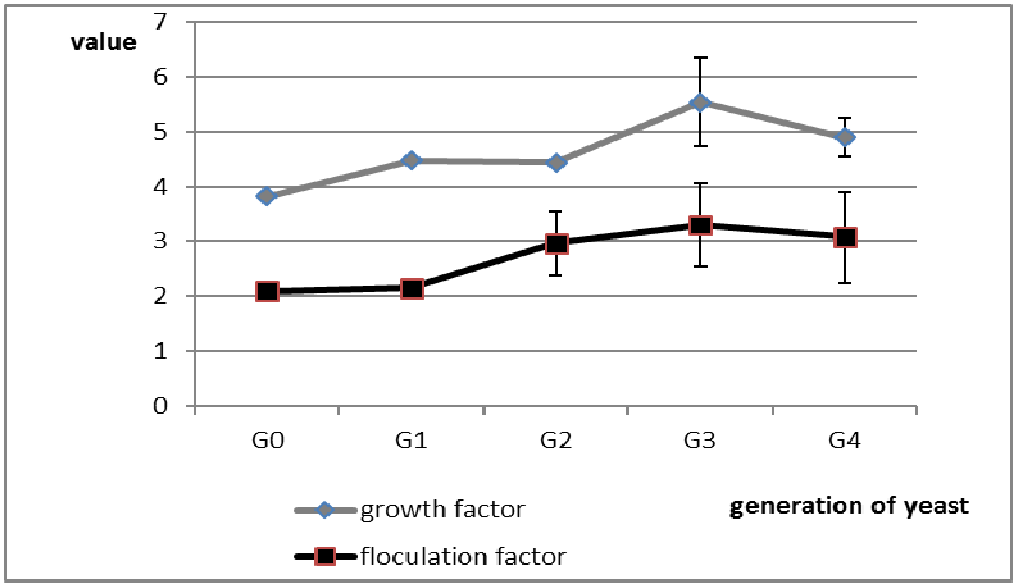

Figure 4 Multiplication factor (Fi) and Flocculation factor (Fi') of yeast in fermenting wort

4. CONCLUSION

From the present study, we can conclude that yeast (*Saccharomyces carlbergensis*) was found to reduce diacetyl to below the threshold level of 0.15 ppm. Furthermore the yeast showed interesting multiplication and flocculation properties. In order to further improve production efficiency, it will be suitable to undertake further studies on the mechanisms available to the yeast cell to reduce diacetyl because a broader knowledge of the diacetyl reduction mechanism in yeast cells could lead to huge time savings at the brewery.

5. ACKNOWLEDGEMENTS

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