Research concerning the use of encapsulated Maturex for beer fermentation

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Abstract
Beer's fermentation utilizes the ability of yeasts to convert sugar into ethanol and carbon dioxide as major products of metabolism. The yeast also produces a series of minor metabolites such as esters, carbonyl compounds, higher alcohols and acids. Diacetyl reduction is the limiting step of beer fermentation. That's why ALDC became available in 1991 and many breweries use it for accelerate beer's maturation by restricting diacetyl formation. In this paper we studied the effects of ALDC use, as well as the use of encapsulated enzyme on beer's aroma.

Keywords: Maturex, enzyme encapsulation, beer's flavour, beer's fermentation.

Resumé
La fermentation de la bière utilise la capacité de la levure de transformer les sucres en etanol et carbon dioxide, les majeurs produits de metabolism. La levure produce aussi une série de mineur metabolites comme des esters, des composés carbonylique, des alcools supérieurs et des acids. La reduction de diacetyl est l'étape limitative de la fermentation de la bière. C'est pour quoi en 1991 ALDC est devenu disponible et plus de brasseries l'utilise pour accélérer la maturation de la bière, par la restriction de la formation de diacetyl. Dans cet article nous avons étudié les effets de l'emploi de ALDC et l'emploi de la enzyme encapsulé sur l'arome de la bière.

Mots clé: Maturex, l'encapsulation de l'enzyme, l'arome de la bière, la fermentation de la bière.

1. Introduction

Fermentation is the most important step in the production of beer. During this process the yeast cells use the nutrients found in wort for growth and metabolism. The main products of this fermentative metabolism are ethanol and carbon dioxide. In addition, the wort fermentation generates a multitude of other minor products of metabolism that contribute to beer's flavour.

The action of yeast on wort is also to remove some components undesirable in beer. The main organoleptic substances in beer are the carbonyl compounds, the organic acids, fusel alcohols and esters. (Briggs, 2004).

The most important carbonyl compound in beer is the diacetyl because it has a low flavour threshold and imparts to beer a butter-like aroma.

The removal of diacetyl from beer is the limiting step of the fermentation process.

The diacetyl is formed by the oxidative decarboxylation (a slow, non-enzymatic reaction) of α-acetolactate secreted by yeast during the synthesis of valine and leucine and it is removed later in the fermentation process by reductive conversion to acetoine and further to 2, 3-butanediol (figure 1). (Hannemann, 2002).

Because the removal of diacetyl is important in achieving an acceptable beer flavour and an optimization and reduction of maturation time is desired, researchers suggested using microbian

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ALDC (α-acetolactate decarboxylase) to convert α-acetolactate directly to acetoine, bypassing the diacetyl stage (figure 1).

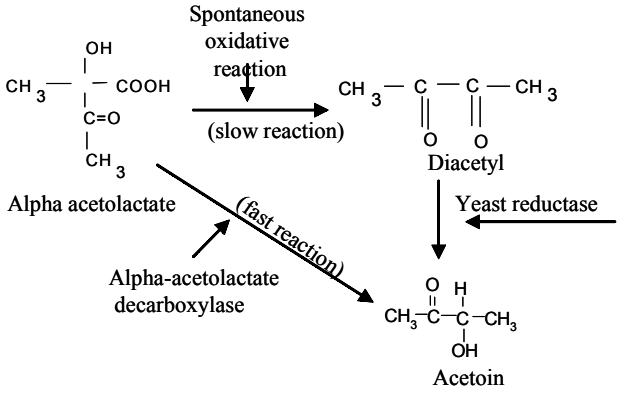

Figure 1. Formation and removal of diacetyl. (Hannemann, 2002)

ALDC became available in 1991, is commercialized under the trade name of Maturex L, and is used by many breweries to accelerate beer maturation. (Hannemann, W.)

Enzyme preparations are not stable for a long period of time and they need special storage conditions. We have tried to study, in this paper, the impact of enzyme encapsulation on beer fermentation process, especially on the most important flavour substances content.

The immobilization of active biological substances has become a universal tool in biotechnology over the past decades. Immobilization can be defined as a procedure that confines substances or cells inside a given system and limits its free diffusion or migration. (Flickinger and Drew, 1999)

Microencapsulated systems were successfully used in food industry and other industries too because of their advantages like a better stability of the enzyme, an enhanced activity, improved selectivity, safer use. These advantages may be different from enzyme to enzyme, from application to application and from carrier to carrier.

2. Materials and methods

For experiment we used synthetic wort with the following composition (for 100 ml): glucose-8g, yeast extract-0.65g, $(NH_4)_2SO_4$-0.26g, KH_2PO_4-0.272g, $MgSO_4$-0.05g, $CaCl_2$-0.05g, $ZnCl_2$-0.042mg, citric acid-0.15g, sodium citrate-0.6g. The medium was sterilized at 121°C for 15 minutes. The glucose solution was sterilized separately avoiding Maillard reaction.

Yeast biomass needed for pitching was obtained by streaking *Saccharomyces carlsbergensis* cells on synthetic medium with 2% agar at temperature of 28°C for 4 days. To slant cultures we added 5 ml of synthetic medium and the cells were brought into suspension. The samples were inoculated with 15×10^6 cells/ml.

Maturex L is a brown liquid containing ALDC produced by a Bacillus subtilis strain. It has a specified activity of 1500 acetolactate decarboxylase units (ADU)/g. We added 3 mg of Maturex to 200 ml wort.

For enzyme encapsulation we used alginate solution of 4% and the droplets were formed in a $CaCl_2$ solution of 50 mM. The encapsulated enzyme was added to obtain the same concentration in the wort like in the samples with free enzyme.

The laboratory apparatus used was:
- Karl Zeiss Jena Microscope – for cell counting,
- Analytical balance Owalabor type 750.05 – for weighing the samples,
- Perkin Elmer gas-cromatograph with cappilary column Chromopack 7773, length 50 m, liquid phase CP WAX , detectors FID and ECD, mobile phase N_2/H_2 – for the determination of aroma compounds,
- Anton Paar DSA 5000 – for alcohol content and extract determination.

The methods used were:
- Direct counting of microorganisms with Thomas camera,
- Gas-chromatographic determination of aroma compounds using EBC method,
- Apparent extract determination with Anton Paar,
- Ethanol determination using standardized method SR 13355-3/1999.

3. Results and discussion

The fermentation process was performed in Erlenmeyer flasks. These were filled with 200 ml synthetic wort with original gravity of 8.41°P. The wort was pitched with 15×10^6 cells/ml *Saccharomyces cerevisiae* yeasts. The fermentation

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process was conducted at a constant temperature of 22˚C. The samples were weighted every 12 hours for establish the end of fermentation. At the end the samples were analyzed for bitterness and polyphenolic content, aroma compounds content, color, pH, apparent extract, alcohol content. The samples were: M-samples without Maturex, for control, ML-the samples with liquid Maturex and MI-samples with encapsulated Maturex.

The apparent extract is varying but not in excess, as it can be seen in figure 2. It has a minimum value for the samples containing free enzyme. Its maximum value is for control samples.

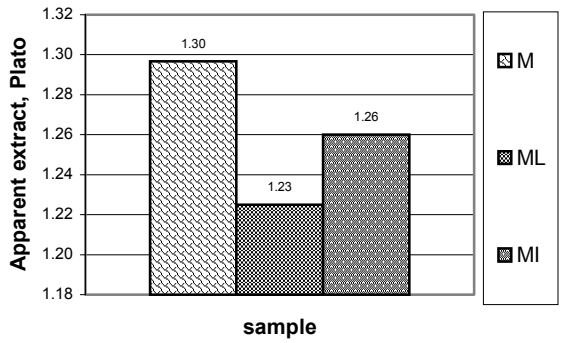

Figure 2. The apparent extract variation.

The most important variations were for aroma compounds. The vicinal diketones content should be lower in the samples containing Maturex because the ALDC from Maturex converts the α-acetolactate directly to acetoine without producing diacetyl. The same enzyme converts also α-acetohydroxy-butyrate to 2, 3-pentandione (Hannemann, 2002). That happened in our trials too as it can be seen in figures 3 and 4.

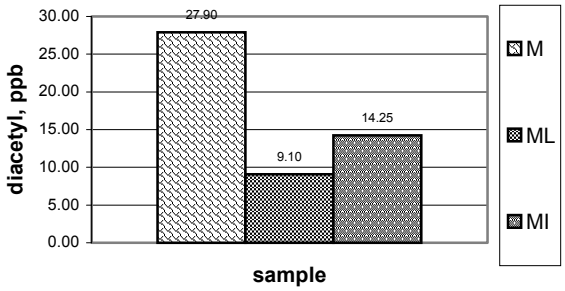

Figure 3. The diacetyl content of the samples.

As figure 3 shows, the samples without Maturex have a higher content of diacetyl. The diacetyl content is higher in the samples with encapsulated enzyme, probably because the ALDC wasn't liberated in sufficient amount from the carrier material. From figure 4 it can be observed that the pentanedione content is higher in the samples with Maturex than in control samples. That happened because the ALDC from Maturex converts α-acetohydroxy-butyrate to 2, 3-pentanedione. (Hannemann, 2002)

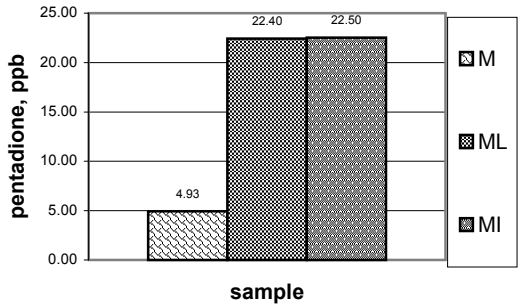

Figure 4. The 2, 3-pentadione content of the samples.

The acetaldehyde content is higher in the samples with free enzyme and it is minimum in the samples with encapsulated Maturex (figure 5).

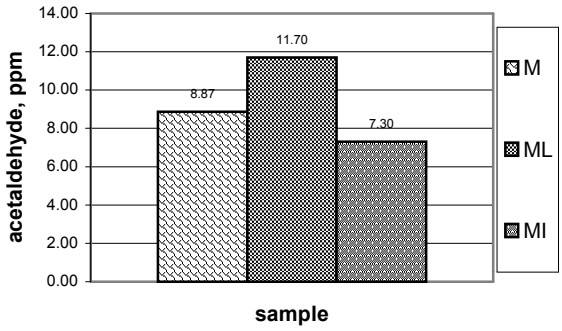

Figure 5. The acetaldehyde content of the samples.

The higher alcohols content differ significant with manner of enzyme adding. For propanol, this can be found in a minimum concentration in the samples with free enzyme and it is maximum in the samples with immobilized enzyme, but the differences are not major (figure 6).

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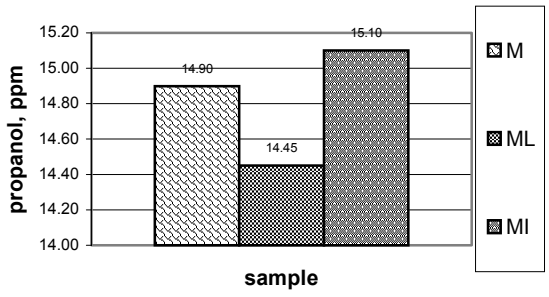

Figure 6. The propanol content of the samples.

The isobutanol content is the same for control samples and the samples with free Maturex as it can be seen from figure 7.

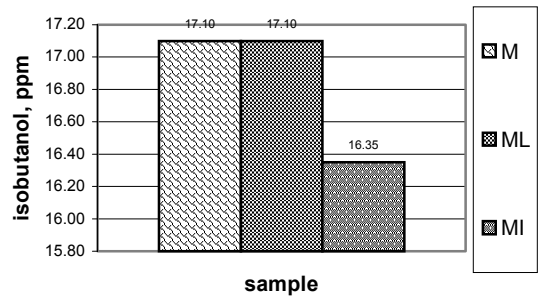

Figure 7. The isobutanol content of the samples.

The isoamyl alcohol content is higher for control samples and has a minimum value for samples with free enzyme. But the differences are not significant (figure 8).

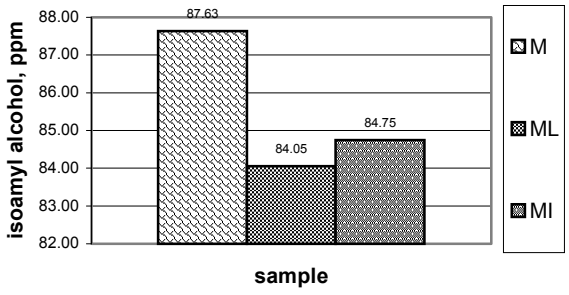

Figure 8. The isoamyl alcohol content of the samples.

The ester content is varying too depending of manner of enzyme adding. It is minimum for the samples with free enzyme, but the differences are not major (figures 9 and 10).

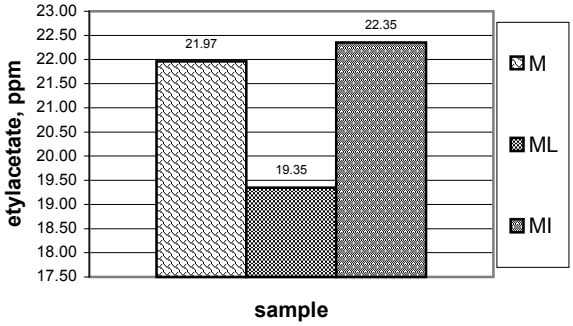

Figure 9. The etylacetate content of the samples.

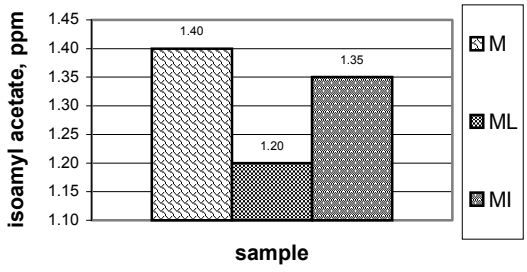

Figure 10. The isoamyl acetate content of the samples.

As Hannemann shows in his report, beers made with and without Maturex are very similar in flavour. Furthermore, beers with ALDC have higher taste scores than those produced without Maturex because of the elimination of diacetyl flavour. As our results show, the aroma substances profile is very similar for the control beer, the beer with free enzyme and the beer with encapsulated enzyme. There are some slightly differences between samples.

4. Conclusions

The use of encapsulated enzymes has become a versatile tool for biotechnology in the last decades because of its benefits. Entrapment of enzymes in a gel matrix of alginates is the most popular system of immobilization reported. Maturex contains an enzyme that is very important for brewing technology. Although many breweries don't use it because of the Reinheitsgebot, the German purity law, the addition of ALDC has no significant effect on fermentation parameters, but in every case limits the amount of diacetyl formed. Furthermore, by adding Maturex the beer process can be shortened

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by 2-3 days without any risk of producing beer with high values of diacetyl.

As our results show, the Maturex do not significant affect the aroma substances content of the beer. The diacetyl content is lower in samples with ALDC. The higher alcohols content and the ester content are, in almost all cases, lower in samples containing ALDC, but the variations are small.

Encapsulated enzyme could be used for beer maturation instead of free Maturex because of the advantages of encapsulation and because of the minor differences between the samples with free and encapsulated Maturex concerning the aroma profile of the final beer.

More research must be made, at pilot scale and full scale trials, for large use of Maturex and, especially of encapsulated Maturex although first results are promising.

5. References

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*** Novo Nordisk Product sheet for Maturex L.