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# Reduction of vicinal diketones by yeast in dependence of carbon dioxide pressure

One of the possibilities how to make beer production more effective is lowering the time of diacetyl and 2,3-pentanedione reduction. These compounds, collectively referred as vicinal diketones, are very important since their taste limits are very low and evolve undesirable change of beer flavour, which is known as buttery, cottage cheese or whey taste. The aim of our work was to study an influence of carbon dioxide pressure during the active carbohydrate fermentation and the end of fermentation on vicinal diketones reduction. We compared vicinal diketones reduction in young beers fermented under carbon dioxide pressure of 100 kPa during the whole main fermentation and in beers fermented without carbon dioxide pressure until extract concentration decreased to 7.5 °P and then under carbon dioxide pressure of 50 kPa. Experiments were done in the wort fermentation of gravity 12.5 °P and 13.5 °P. Lower carbon dioxide pressure accelerated vicinal diketones reduction and speeded up the end of fermentation. Wort concentration had no influence on vicinal diketones reduction in studied wort gravities (12.5 °P and 13.5 °P).

BC 23 Fermentation

(Descriptors: Beer, vicinal diketones, diacetyl, 2,3-pentanedione, carbon dioxide pressure.

Deskriptoren: Bier, Vicinale Diketone, Diacetyl, 2,3-Pentandion, Kohlendioxiddruck)

## 1 Introduction

Yeasts produce  $\alpha$ -acetolactate and  $\alpha$ -acetohydroxybutyrate, precursors of vicinal diketones, as they synthesise the amino acids valine and leucine needed for protein synthesis.  $\alpha$ -acetolactate and  $\alpha$ -acetohydroxybutyrate are excreted into the fermenting wort and converted nonenzymatically to 2,3-butanedione (diacetyl) and 2,3-pentanedione, collectively referred as vicinal diketones (Fig. 1). Towards the end of fermentation vicinal diketones are subsequently re-assimilated by the yeast and reduced enzymatically to butanediol and pentanediol [1,2,3,4].

Yeast strains vary in their capacity to excrete the  $\alpha$ -acetolactate and certain fermentation conditions can enhance the production of this precursor [5].

Flavour limit of vicinal diketones was determined on 0.1 mg/l and these compounds have big importance on taste. Above the flavour-threshold-level they cause an undesirable change of beer taste, which is known as a buttery, cottage cheese or whey flavour [6]. Butanediol and pentanediol, as well as acetoin are sensorially non-active.

The breakdown of these vicinal diketones occurs parallel to other maturation reactions during the beer conditioning process and is therefore nowadays regarded as the essential criterion for the state of maturation of a beer [7]. Production of  $\alpha$ -acetolactate is limited by lower pH-values of beer (under 4.6) but higher top-pressure during fermentation supports its production [8].

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The mechanism of vicinal diketone production was clarified already a long time ago [9]. It is known that production and subsequent re-assimilation of vicinal diketones are determined by three steps:

- synthesis of  $\alpha$ -acetohydroxy acids;
- oxidative decarboxylation of  $\alpha$ -acetohydroxy acids to their diketones;
- vicinal diketones reduction by yeasts.

Conversion of  $\alpha$ -acetohydroxy acids to vicinal diketones is purely a chemical step and is the slowest or rate-limiting. Efficient regulation of diacetyl and 2,3-pentanedione content can be reached by prevention of their precursors production, or by accelerating the rate of chemical decarboxylation.

Typically only light counterpressure of carbon dioxide is applied to fermentation vessels. The use of carbon dioxide top-pressure of up to 200 kPa has an important influence on fermentation and beer characteristics. Brewers have used higher temperatures to increase fermentation rate, decreasing overall time, but they have found that excessive amounts of volatiles were formed. Fermentation under carbon dioxide pressure was found to retard yeast

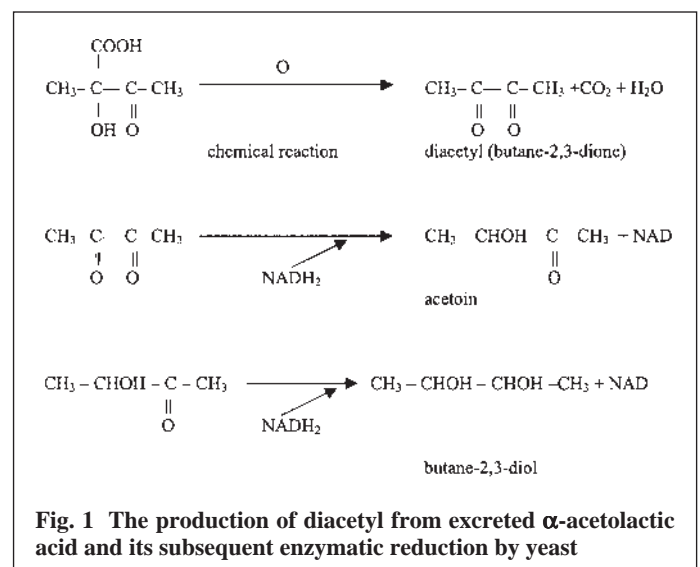
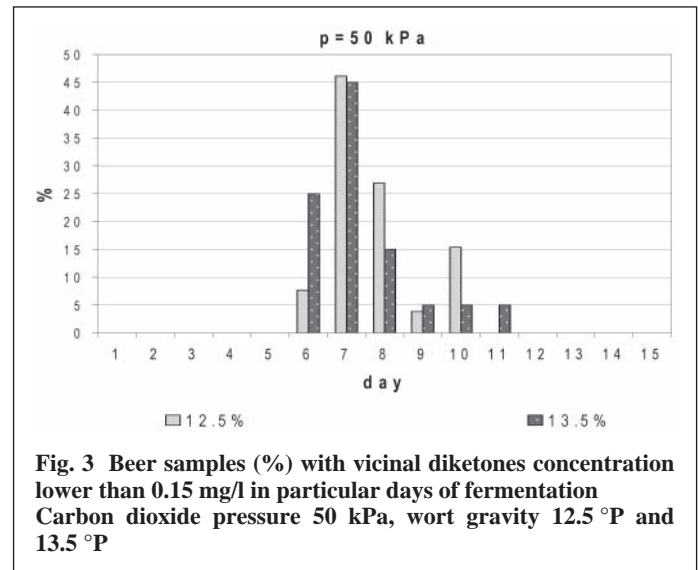
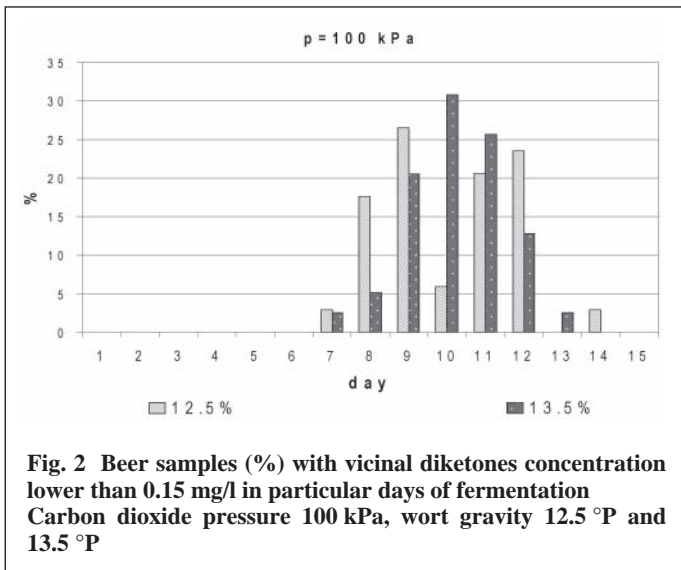


Fig. 1 The production of diacetyl from excreted  $\alpha$ -acetolactic acid and its subsequent enzymatic reduction by yeast



growth and volatile formation. Carbon dioxide pressure appears to have an insignificant effect on the maximum concentration of vicinal diketones precursors or the conversion rate to vicinal diketones. However, the longer fermentation time under pressure allows more time for the conversion of precursors and subsequent removal of vicinal diketones by yeast [10].

The aim of this work was to study how lower carbon dioxide pressure affects the rate of vicinal diketones reduction during the main fermentation or in the end of fermentation.

## 2 Materials and methods

### 2.1 Microorganisms

We used an operation strain of brewer's yeasts *Saccharomyces cerevisiae* subsp. *uvarum* W 34/70. The culture was kept on slanting wort agar at 4 °C. Every three months it was pre-inoculated.

### 2.2 Wort fermentation

Pitching temperature was 8.5 °C and during primary fermentation it was kept at 14 °C. Fermentation process was carried out under two different degrees of carbon dioxide pressure:

- Whole primary fermentation was run under carbon dioxide overpressure of 100 kPa.
- Fermentation run without carbon dioxide counterpressure until extract concentration decreased to 7.5 °P. Then pressure was increased to 50 kPa and maintained at this value. Primary fermentation in both cases ran in cylindro-conical tanks (CCT) with volume 2170 hl, filled on 83% using wort of gravity 12.5 °P and 13.5 °P. Beer maturation was carried out in horizontal vessels.

The fermentation experiments were carried out 34 times at pressure 100 kPa using 12.5 °P wort, 39 times at pressure 100 kPa using 13.5 °P wort, 20 times at pressure 50 kPa using 13.5 °P wort and 26 times at pressure 50 kPa using 12.5 °P wort.

Vicinal diketones concentration was measured after harvesting the main yeast every day until their concentration decreased to the required level of 0.15 mg/l.

### 2.3 Analytical methods

Vicinal diketones concentration was determined as total vicinal diketones after distillation with water stream by the EBC recommended spectrophotometrical method [11].

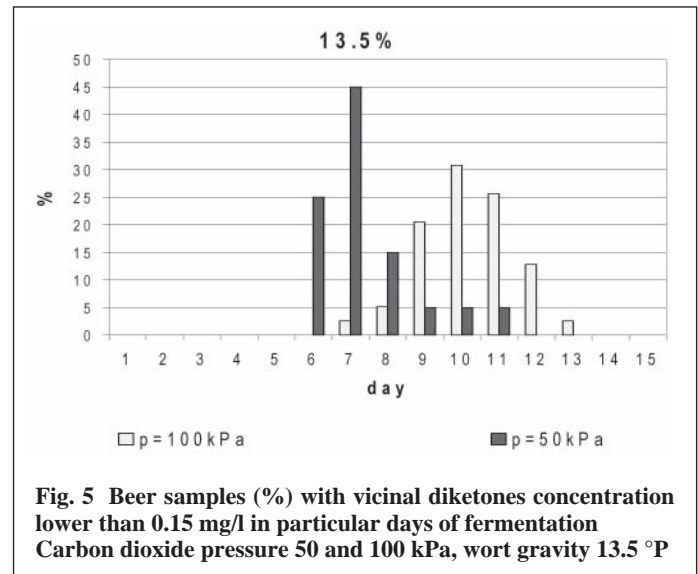
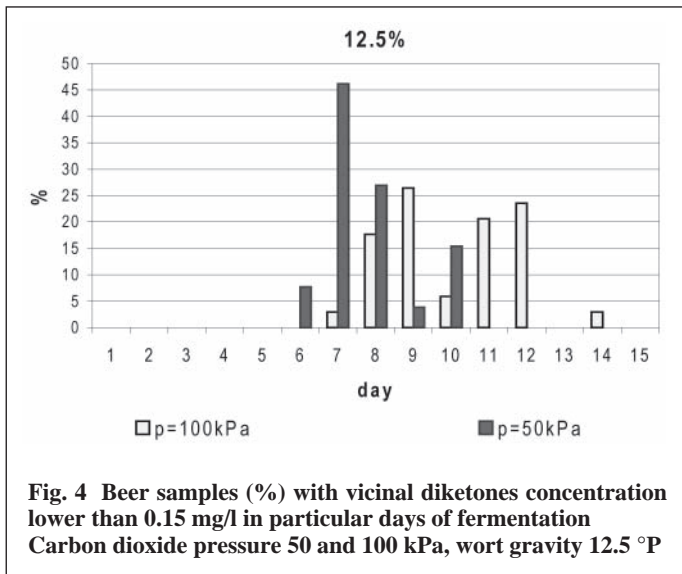
## 3 Results and discussion

Vicinal diketones are undesirable substances of young and ready beer. Their conversion to sensory non-active compounds happens during the end of main fermentation, so fermentation is limited by their reduction under the threshold value – maximum concentration of diacetyl is 0.2 mg/l. Using worts of gravities 12.5 °P and 13.5 °P we compared vicinal diketones reduction in young beers fermented under carbon dioxide overpressure of 100 kPa and vicinal diketones reduction in young beers fermented without carbon dioxide counterpressure until extract concentration decreased to 7.5 °P – then the reduction valve of cylindro-conical tank was closed and the end of primary fermentation was carried out at a top-pressure of 50 kPa.

Vicinal diketones concentration was measured after removing the main yeast harvest. Measurement was repeated every 24 hours until the vicinal diketones concentration decreased to the required level of 0.15 mg/l.

The results quoted were obtained from parallel experiments under each fermentation conditions using both wort gravities (see Material and methods). From total number of fermentation experiments we calculated the percentage of beer samples in which concentration of vicinal diketones dropped at the required value of 0.15 mg/l in particular day of fermentation.

We supposed that we would find some differences in vicinal diketones reduction in dependence on wort gravity, but it was not confirmed, the differences were not significant. Under the pressure of 100 kPa, vicinal diketones concentration started to drop to the required level of 0.15 mg/l in some beer samples on the seventh day of fermentation using wort of both gravities (Fig. 2). Vicinal diketones reduction was finished on the thirteenth day of fermentation using wort of gravity 13.5 °P and on the fourteenth day using wort of gravity 12.5 °P. Under pressure of 50 kPa, in both cases vicinal diketones concentration started to drop to the required level on the sixth day and was finished on the tenth day of fermentation using wort of gravity 12.5 °P and on the eleventh



day of fermentation using wort of gravity 13.5 °P (Fig. 3). Significantly slower reduction of vicinal diketones was found when the fermentation was carried out under higher carbon dioxide overpressure – 100 kPa (Fig. 4 and Fig. 5).

#### 4 Conclusions

We studied the possibility of faster vicinal diketones reduction under unusual fermentation conditions in worts of different gravity. The hypothesis that lower carbon dioxide pressure in the last stage of fermentation can improve reduction of vicinal diketones was confirmed. Fermentation under a reduced carbon dioxide pressure of 50 kPa only at the end of fermentation accelerated reduction of vicinal diketones compared to fermentation under a constant carbon dioxide pressure of 100 kPa. Lower carbon dioxide pressure had no influence on beer quality, foaming or carbon dioxide content in the finished product.

Wort concentration had no influence on vicinal diketones reduction in the studied wort gravities (12.5 °P and 13.5 °P).

#### 5 Zusammenfassung

Šepeřová, G., Cvengrošková, M. und Šmogrovičová, D.: Reduktion vicinaler Diketone durch Hefe in Abhängigkeit vom CO<sub>2</sub>-Druck — Monatsschrift für Brauwissenschaft 56, Nr. 3/4, ?? – ??, 2003

#### BC 23 Gärung

Eine Möglichkeit, die Bierherstellung effizienter zu gestalten, ist die Verringerung der Zeitdauer für die Reduktion von Diacetyl und 2,3-Pentandion. Diesen Verbindungen, allgemein bekannt als Vicinale Diketone, kommt eine sehr große Bedeutung zu, da ihr Geschmacksschwellenwert sehr niedrig ist und sie nicht wünschenswerte Geschmacksveränderungen hervorrufen, die an Butter, Hüttenkäse oder Molke erinnern. Das Ziel unserer Arbeit bestand darin, den Einfluss eines CO<sub>2</sub>-Drucks, sowohl während der aktiven Kohlenhydrat-Fermentation als auch am Ende der Gärung hinsichtlich der Reduktion Vicinaler Diketone zu untersuchen. Wir verglichen die Reduktion Vicinaler Diketone in Jungbieren, die unter einem CO<sub>2</sub>-Druck von 100 kPa vergoren wurden, über die gesamte Hauptgärung hinweg, sowie in Bieren, die ohne CO<sub>2</sub>-Druck vergoren wurden, bis die Extraktkonzentration auf 7,5°Plato abgenommen hatte. Daran anschließend erfolgte die entsprechende Beurteilung bei einem CO<sub>2</sub>-Druck von 50 kPa. Die Gärungsversuche wurden mit Stammwürzen von 12,5 und 13,5°Plato durchgeführt. Ein niedrigerer CO<sub>2</sub>-Druck beschleunigte die Reduktion Vicinaler Diketone und führte zu einer kürzeren Gärdauer. Die Würzekonzentration beeinflusste die Reduktion der Vicinalen Diketone bei den untersuchten Stammwürzen (12,5 und 13,5°Plato) nicht.

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Šepeřová, G., Cvengrošková, M. et Šmogrovičová, D.: Réduction des dicétones vicinales par la levure en fonction de la pression du CO<sub>2</sub> — Monatsschrift für Brauwissenschaft 56, No. 3/4, ?? – ??, 2003

#### BC Fermentation

Pour rendre la fabrication de bière plus efficace, il existe la possibilité de diminuer le temps de réduction du diacétyle en 2,3-pentanedione. Les dicétones vicinales sont des composés bien connus et leurs seuils de perception sont très bas. Elles ont une grande importance dans le changement de saveurs indésirables rappelant celles du beurre, du fromage des alpes ou du lactosérum (petit lait). Le but de ce travail était d'examiner l'influence de la pression du CO<sub>2</sub> sur la réduction des dicétones vicinales aussi bien pendant la fermentation active des glucides qu'à la fin de la fermentation. Nous avons comparé la réduction des dicétones vicinales pendant toute la fermentation principale dans les bières vertes qui ont été fermentées à une pression de 100 kPa, avec des bières qui ont été fermentées sans pression de CO<sub>2</sub>. Ces fermentations ont été suivies jusqu'à une diminution de l'extrait à 7,5°Plato. A la suite des essais, une évaluation correspondant à 50 kPa de pression du CO<sub>2</sub> a été effectuée. Les essais de fermentation ont été réalisés avec des moûts d'une densité de 12,5° et 13,5°Plato. Une pression inférieure du CO<sub>2</sub> augmentait la vitesse de réduction des dicétones vicinales et conduisait à des durées de fermentation plus courtes. La densité du moût (12,5 et 13,5°Plato) n'influençait pas la réduction des dicétones vicinales.

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