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Activation of soluble-inactive limit dextrinase and its application

Most limit dextrinase exists usually in the inhibited form following malting. We explored the transformation of this inhibited enzyme to the free and uninhibited enzyme by the control of aeration in germination, and compared the results to those of malt that was aerobically germinated and that was stored before beer making. It was shown that there was potential to improve the utilization level of soluble-inactive limit dextrinase and to receive other benefits as well.

BC 21 Maltings

(Descriptors: soluble-inactive limit dextrinase, activation, germination.

(Deskriptoren: Lösliche-inaktive Grenzdextrinase, Aktivierung, Keimung).

1 Introduction

Limit dextrinase (EC3.2.1.4) is commercially important because there is evidence that conditions during mashing which promote activation of this enzyme lead to an alteration of carbohydrate composition that increases fermentability (1); the free enzyme hydrolyzes α-1,6 glucosidic linkages in amylopectin and branched dextrins, leading to an increase in fermentable carbohydrates in wort. The activity of limit dextrinase is minimal in mature barley grains, but during germination its activity slowly increases after an initial lag period (2). In germinating stage, limit dextrinase exists in three forms: free (soluble-active), latent (soluble-inactive) and bound (3). Our aim was to explore the activation of soluble-inactive limit dextrinase during malting in order to maximize its potential activity during mashing. The latent limit dextrinase should be active particularly during the early stages of mashing because there is evidence that malt extract protects the enzyme (4).The activation is very important because 90 % of the extract in wort are carbohydrates consisting of 75 % of fermentable sugars and 20 % of non-fermentable dextrins (5). Greater limit dextrinase activity will decrease the non-fermentable proportion of the carbohydrate and obviously shorten malt storage period before beer making.

The specific aims laid down in this paper were to determine

- the optimum germination regime under aerobic and anaerobic conditions in a 5-day germination process to obtain a high free activity of limit dextrinase, and
- whether malt storage period could be shortened by activation of latent limit dextrinase during malting.

2 Experimental material

Australian barley Stirling was supplied by Guangzhou Malting Co. Ltd.

Chemicals were of the highest quality available.

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Germination of barley at 16 – 17°C for 120hrs was carried out in a Seeger micro-malting plant. The steeping regime was 5hrs steeping, 10hrs air rest, 4hrs steeping, 9hrs air rest and 1hr steeping at 18 – 19°C. Germination of barley under anaerobic conditions was achieved by transferring grain into 100ml plastic bags after having germinated under aerobic conditions for some time, then sealed and incubated at 16 – 17°C. The samples were dried before analysis always by the same continuous kilning process #4 (30° C for 0.5hr, 50° C for 8hrs, 60° C for 1hr, 70° C for 2 hrs, 82° C for 2hrs). Grain was germinated for 5 days, with subsamples transferred every 10hrs to anaerobiosis during the germination period.

3 Limit dextrinase extraction and assay

Limit dextrinase was extracted and analyzed according to literature (6), (7) and (8).

4 Results and discussion

Malts were prepared by germination under aerobic conditions for 5 days. The free and the total of limit dextrinase was determined in the malt from each 10hr subsample (Fig. 1). A maximum of approximately 500mU/g was achieved for the total of limit dextrinase after 5 days of germination, with free activity constituting 10 – 16% of this total activitiy. The free limit dextrinase activity measured in each 10hr sample of the 5 days never exceeded 82mU/g (Fig. 1). This illustrates the lack of free limit dextrinase activity that is found under aerobic malting conditions.

It is known that under anaerobic conditions a malt can be produced that has a high free limit dextrinase activity. Experiments were therefore performed to find a condition to increase the level of free enzyme activity. After steeping, barley grains were transferred to anaerobiosis. The samples transferred after 10hrs aerobic germination received 110hrs of anaerobiosis, whereas the samples transferred after 110hrs aerobic germination received only 10hrs of anaerobiosis. The free and the total of limit dextrinase is shown in Fig. 2.

The malts having germinated under aerobic conditions followed by anaerobic conditions showed a general increase in total limit dextrinase activity with increasing length of aerobic germination (Figs. 1 and 2). The total (overall) activity of limit dextrinase kept increasing during germination. Since the free enzyme activity never exceeded the overall activity it can be concluded that there is activation of limit dextrinase when germination takes place under anaerobic conditions and that there is no further synthesis of the enzyme. The free enzyme activity reached a plateau with 60hrs germination under aerobic conditions (Fig. 2). There was a sharp decrease when anaerobic germination was less than 40hrs.

For comparing the influences of storage period on free and total limit dextrinase, barley malt grains having germinated under aerobic conditions were fully put into 50ml plastic bags, tightly sealed in a 2 litre plastic box and stored at 20°C. A plastic bag of malt sample was analyzed that was taken from the plastic box every 10 days. The patterns of the activation of limit dextrinase during anaerobic storage period showed that obviously the free enzyme increased slowly, reached the equivalent level to the plateau in Fig. 2 after 50 days storage, and kept increasing as storage continued. (Fig. 3) There was no highlighted variability for total limit dextrinase among the samples during storage , and this also applied for the total limit dextrinase between the malt samples before storage and those after storage. The results indicated that the maximum potential of the malt product was under the control of the malt following malting. Tests of activation and storage were repeated. It was found that the patterns were almost similar though there was variability of data between the barley varieties.

5 Conclusion

It was shown that there was potential for malting industries and breweries to enhance the utilization of soluble-inactive limit dextrinase and remarkably shorten malt storage period, cut down cost, make more profit and win more active competition opportunities.

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6 Zusammenfassung / Resumé

Huang, X.: Aktivierung löslicher inaktiver Grenzdextrinase und ihre Anwendung— Monatsschrift für Brauwissenschaft 56, No. 7/8, 132 – 133, 2003

BC 21 Mälzerei

Der Großteil der Grenzdextrinase liegt normalerweise nach dem Mälzen in inhibierter Form vor. Wir haben die Transformation dieses inhibierten Enzyms zum freien und nicht-inhibierten Enzym mittels Kontrolle der Belüftung während des Keimens untersucht und die Ergebnisse mit denen eines Malzes verglichen, das während der Keimung belüftet worden und vor dem Verbrauen gelagert worden war. Es konnte gezeigt werden, dass ein Potential zur Verbesserung der Verwertbarkeit der löslichen, inaktiven Grenzdextrinase besteht und noch andere Vorteile zu erzielen sind.

Huang, X.: Activation de dextrinases limites inactives et leur utilisation — Monatsschrift für Brauwissenschaft 56, No. 7/8, 132 – 133, 2003

BC 21 Malterie

La majorité des dextrinases limites est normalement sous forme inactive après le maltage. Nous avons examiné la transformation de cette enzyme inhibée en enzyme libre et non inhibée par un contrôle de l'aération pendant la germination. Les résultats ont été comparés avec ceux d'un malt qui n'a pas été aéré pendant la germination et qui a été stocké avant le brassage. On a pu montrer, qu'il existe un potentiel d'amélioration dans l'utilisation de dextrinases limites solubles et inactives. D'autres avantages sont attendus.

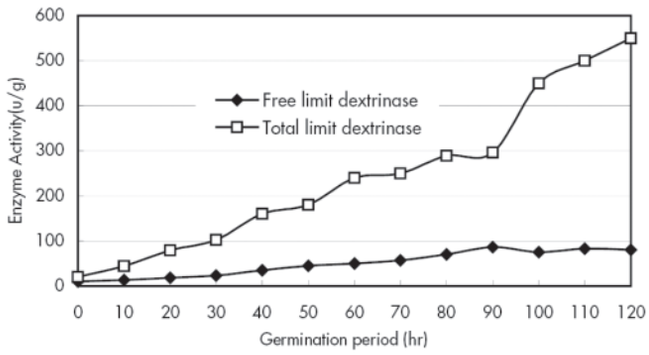

Fig. 1 Free limit dextrinase activity under aerobic malting conditions

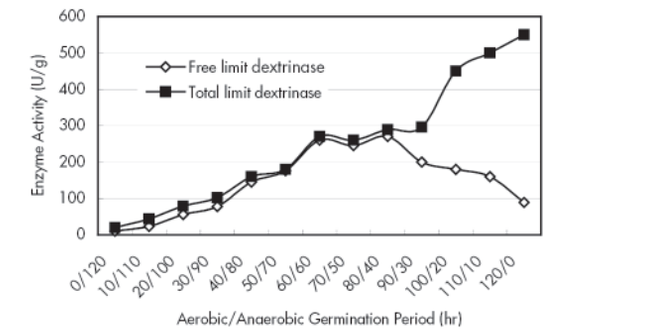

Fig. 2 Free limit dextrinase activity under anaerobic malting conditions

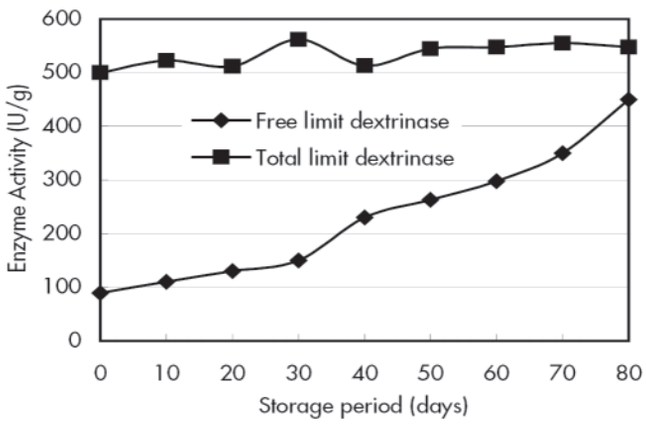

Fig. 3 Activation of limit dextrinase during anaerobic storage period

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