# Effect of Anaerobic Treatment on Carbohydrate-Hydrolytic Enzyme Activities and Free Amino Acid Contents in Barley Malt

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## ABSTRACT

**Effects of acute anoxia on carbohydrate hydrolytic enzyme activities and free amino acid contents in malt were examined. Malts were prepared with barley grains germinated for 7 days which contained the highest levels of amylolytic and(1-3,1-4)-$\beta$-glucanase activities. $\alpha$-Amylase and $\beta$-amylase activities in malts were not significantly affected by anoxia for 5 or 10 h.(1-3,1-4)-$\beta$-Glucanase activity, however, decreased about 7 to 10% by anoxia for 5 or 10 h. Alanine and $\gamma$-aminobutyric acid content changed drastically. Alanine contents in malts increased by 2.2- and 2-fold, and $\gamma$-aminobutyric acid contents by 1.4- and 1.9-fold under anoxia for 5 and 10 h, respectively.**

**Key words: barley, malt, anoxia, amylases,(1-3,1-4)-$\beta$-glucanase, $\gamma$-aminobutyric acid(GABA).**

Oxygen is required absolutely as a terminal electron acceptor during respiration in higher plants. Oxygen deficits, consequently, cause drastic changes in metabolism and growth and development of plants. The seed of most higher plants, except rice and *Echinochlora crusgalli*, are unable to germinate in anoxia (Al-Ani et al., 1985; Alpi and Beevers, 1983). Barley, wheat, oat and rye seeds fail to produce $\alpha$-amylase under anoxia, while rice seeds synthesize the enzyme (Perata et al., 1993). Under anoxia glycolysis and alcoholic fermentation undergo to recycle NADH and to produce ATP. Therefore, higher fermentative metabolism is important for plant tolerance to anoxia (Raymond et al., 1985). Even though the metabolic response and adaptation of plants to chronic anaerobic conditions has been examined extensively, there are few reports on the effect of short-term anoxia on plant metabolism.

In fruit, however, short-term anoxia or hypoxia has been proven to be beneficial for fruit quality improvement. Low oxygen concentrations have been widely used in controlled atmosphere storage of harvested fruit with the goal of prolonging fruit shelf-life (Knee, 1991). Short-term anoxic treatments of harvested fruit improve fruit aroma and quality (Pesis, 1995).

Amino acid metabolism of higher plants also changes drastically under short-term anoxia. Alanine and $\gamma$-aminobutyric acid (GABA) increase rapidly in tissues under anoxia (Streeter and Thompson, 1972; Yun and Lee, 1995; Yun and Yoo, 1996). For the utilization of the hypertensive effect of GABA, specialty teas containing higher levels of GABA are produced with tea leaves treated under anoxia for a short period (Omori et al., 1987; Yun et al., 1995).

Germinated barley grains are used to prepare malt which is a primary material for brewing and fermentative food and beverage industries. Demand for malt for the production of Sikhe, a traditional Korean beverage, has recently increased enormously in Korea. Quality of Sikhe is most heavily influenced by the quality of malt (Suh et al., 1997). However there are few studies on malt production and quality improvement (Suh et al., 1997).

In this study, the effects of short-term anoxia on carbohydrate-hydrolytic enzyme activities and amino acid content in malt were examined.

## MATERIALS AND METHODS

### Plant materials

Barley grains (*Hordeum vulgare* L. cv. Olbori) produced in the 1996 growing season at the farm of Chonbuk National University were used for this experiment. Barley grains were allowed to germinate from 0 to 9 days at 15℃ after surface-sterilization and imbibition in a solution containing antibiotics (Yun et al., 1994). Grains germinated for 7 days were subjected to an anaerobic treatment with nitrogen gas for 5 or 10 h at room temperature. All samples were frozen immediately after collection and kept at −70℃ for seven days before freeze drying. Freezedried samples were kept at 5℃ until enzyme activity and amino acid analyses.

### Enzyme activity and amino acid assays

Enzyme activities were determined using commercially available dye-labeled substrates (Megazyme, Ireland). $\alpha$-Amylase and $\beta$-amylase activities were measured according to McCleary and Sheehan (1987) and McCleary and Codd (1989) using blocked *p*-nitrophenyl maltoheptaoside and *p*-nitrophenyl maltopentaoside as $\alpha$-amylase and $\beta$-amylase substrates, respectively. (1-3,1-4)-$\beta$-Glucanase activity was assayed using Azo-barley glucan substrate according to McCleary and Shameer (1987). Free amino acids in deproteinated malt samples were analyzed using HPLC system (TSP, USA) supplemented with a postderivatation instrument (PCX3100, Pickering, USA) according to Yun and Lee (1995).

## RESULTS AND DISCUSSION

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In surface-sterilized dry mature grains imbibed for 16 h before the initiation of germination, allowed to germinate for 0, 1, 3, 5, 7, and 9 days, enzyme activities increased rapidly during the early stage of germination (Fig. 1a, b, and c). $\alpha$-Amylase activity was very low in imbibed grains (at 0 day after germination (DAG)) and increased rapidly until the activity reached the highest level at 5 DAG. The activity remained around the highest level until 9 DAG. Higher levels of $\beta$-amylase activity occurred in grains at 3 DAG and the activity increased continuously from 3 to 9 DAG. (1-3,1-4)-$\beta$-glucanase activity increased most rapidly from 3 to 5 DAG and the activity retained its highest level at 7 DAG. The above results indicate that germinating barley grains at around 7 to 9 DAG contain the highest levels of amylolytic and (1-3,1-4)-$\beta$-glucanase activities under germination conditions used in this study. Shoot and root length at 7 DAG were 2.9 and 2.1 cm, respectively (Fig. 1d).

Malts are used as sources of carbohydrates and enzymes in the brewing and fermantation industries. Amylolytic enzyme activities in malt are important in determining the quality of Sikhe (Suh et al., 1997). Therefore, 7 DAG were subjected to an anaerobic treatment for 5 or 10 h to examine the effect of acute anoxia on the enzyme activities and free amino acid contents in malt. Malts were prepared by freeze drying grains immediately after the anaerobic treatment.

Acute anoxia treatment for 5 or 10 h did not affect both $\alpha$- and $\beta$-amylase activities (Fig. 2a, b) in malt. (1-3, 1-4)-$\beta$-Glucanase activity, however, decreased by 6.9% and 10.5% under 5 and 10 hr anoxia, respectively (Fig. 2c).

$\alpha$-Amylase and $\beta$-amylase are not induced in imbibed barley and wheat grains kept under anoxia resulting in failure of germination (Guglielminetti et al., 1995; Perata et al., 1993) probably due to the inability to transcribe the mRNAs for the enzymes in aleurone layers (Perata et al., 1993). There are few reports on the effect of short-term anoxia on the amylolytic enzymes. Levels of enzyme activities are expressed through the multi-step regulation processes. Even though the transcription of mRNAs for the amylolytic enzymes are inhibited by 5 or 10 h anoxic treatment, its negative effects on the enzyme activities may be negligible in 7 DAG grains at their highest enzyme activity levels. Contrary to this, (1-3,1-4)-$\beta$-glucanase activities decreased significantly. Further investigations on the specific degradation and differences in half-life of enzymes under short-term anoxia should be needed to explain the different responses of enzymes to anoxia.

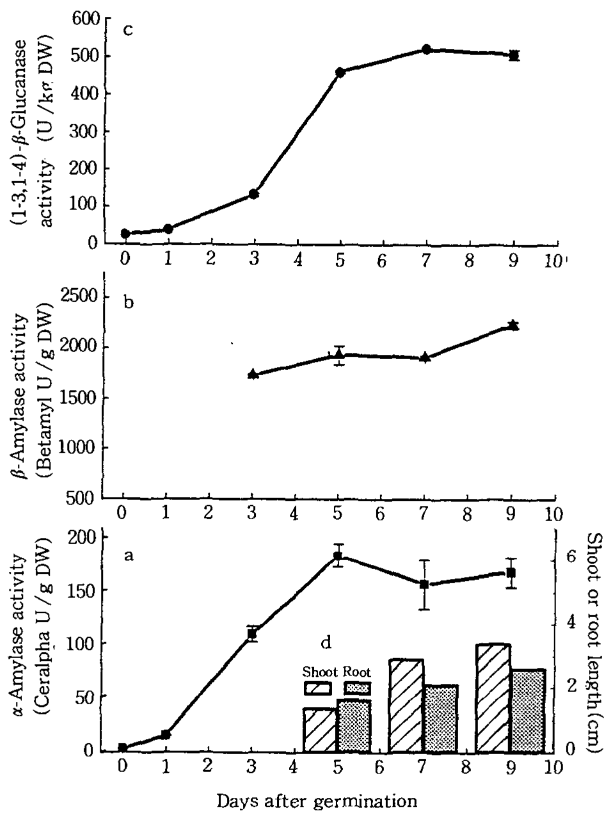


Fig. 1. Changes in activities of $\alpha$- (a) and $\beta$-amylases (b), and (1-3,1-4)-$\beta$-glucanases (c) in germinating barley grains. Shoot and root growth of germinating barley seedlings (d).

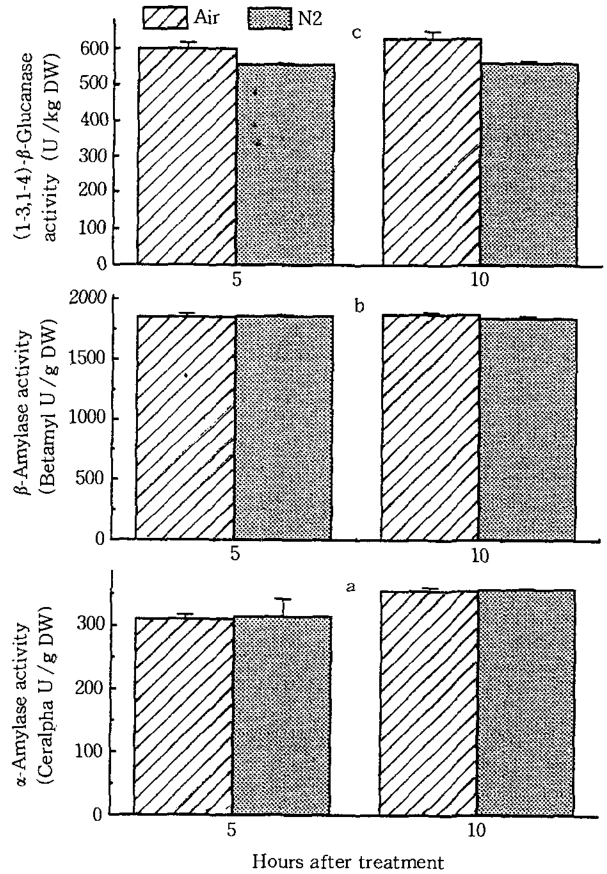


Fig. 2. Effect of anoxia on $\alpha$- (a) and $\beta$-amylases (b), and (1-3,1-4)-$\beta$-glucanases (c) in malts prepared with barley grains germinated for 7 days.

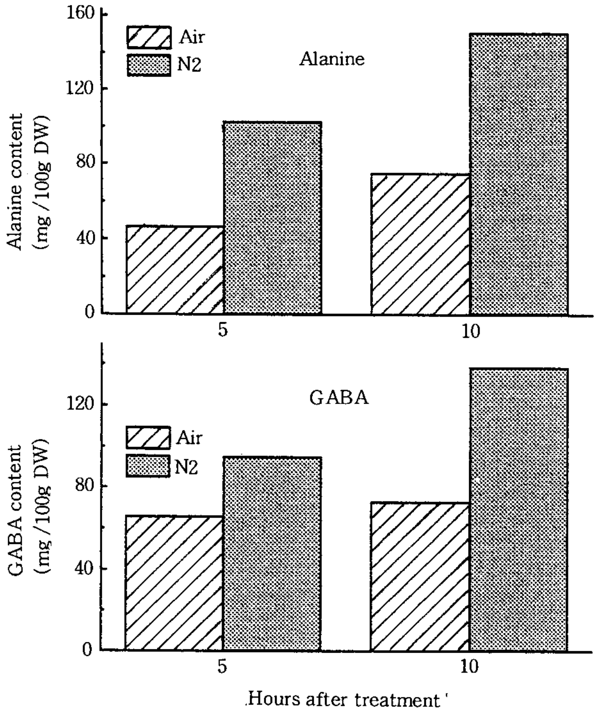


Fig. 3. Effect of anoxia on the contents of alanine and GABA in malts prepared with barley grains germinated for 7 days.

Effect of acute anoxia on two free amino acid contents in malt was also examined. Alanine content increased by 2.2- and 2-fold under 5 and 10 h anoxia treatment. GABA content also increased by 1.4- and 1.9-fold under 5 and 10 h anoxia treatment, respectively (Fig. 3).

Free amino acid content changes drastically in plants under hypoxia or anoxia primarily due to the characteristics of their biosynthetic pathways. Alanine and GABA are the typical amino aicds that increase drastically under anaerobic conditions (Streeter and Thompson, 1972; Yun and Lee, 1995; Yun and Yoo, 1996). Alanine is synthesized by an one-step transamination of glutamate to pyruvate. Pyruvate also increases under anaerobic conditions (Streeter and Thompson, 1972). GABA is produced by the $\alpha$-decarboxylation of glutamate and the activity of glutamate decarboxylase increases under anaerobic conditions (Knight et al., 1991). In this study activation of corresponding enzymes under anaerobic conditions and the one-step conversion process may result in the accumulation of alanine and GABA under 5 or 10 h anoxia.

These results indicated that short-term anaerobic treatments can be used to increase GABA, which has been proven to have a hypertensive effect, in malt without a significant loss of amylolytic enzyme activities.

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