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Effects of steeping and anaerobic treatment on GABA (γ-aminobutyric acid) content in germinated waxy hull-less barley

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ABSTRACT

Effects of steeping conditions (time, temperature and soaking solution) and anaerobic storage on the gamma-aminobutyric acid (GABA) content in waxy hull-less barley grains during germination was examined. The barley kernel was steeped for 16 h at different temperatures (5, 15 or 35 °C) either in water or in a buffer solution (pH 6.0, 50 mmol/L sodium acetate) and then germinated at 15 °C for 72 h. To reach the optimum water content (36–44 g/100 g) for germination, a longer steeping period was required when steeping temperature was lower (16 h at 5 °C vs. 8 h at 15 °C). At 35 °C for steeping, however, the water content in the grains increased excessively, and thus germination percentage became much less than those at 5 and 15 °C. The GABA content increased with increasing germination time and was higher in the buffer solution than water. These findings indicate that the glutamate decarboxylase (GAD), which is the rate-limiting enzyme for GABA synthesis, is more activated by extending germination at controlled pH (6.0). An anaerobic storage with nitrogen in the dark for the germinated barley grains substantially raised the GABA content: 14.3 mg/100 g after the treatment for 12 h, which was four times higher than that of control sample (3.7 mg/100 g). Overall results suggest that the steeping prior to germination greatly affects the GABA production during the germination of barley, and the anoxia storage with nitrogen after the germination increases the GABA content.

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the major cereal grains consumed worldwide and has the ability to grow in diverse environments (McIntosh, Le Leu, Kerry, & Goldring, 1993). The demand for barley in human diets is continually increasing (Edney, 1996) because of the health benefits associated with the dietary fiber in barley, such as lowering plasma cholesterol (Newman, Lewis, Newman, & Boik, 1989) and reducing glycemic index (Jenkins, Jenkins, Zdravkovic, Würsch, & Vuksan, 2002). Hull-less barley contains more protein, starch, and total and soluble β-glucan than hulled barley. In contrast to hulled barley, hull-less barley kernels lose their hull during threshing, making pearling unnecessary, which results in benefits for food uses (easy to mill, no loss of nutrients due to pearling) (Berglund, Fastnaught, & Holm, 1992). Waxy hull-less barley is a unique type of barley and has received attention as potential source of dietary fiber due to its high level of soluble fiber or β-glucan (Berglund et al., 1992).

Barley is the malting grain predominantly used for alcoholic fermentation (Rimsten, 2003). Steeping is widely acknowledged as a critical stage in malting process in order to increase the moisture content of barley grains to promote germination. It allows uptake and distribution of moisture in the kernels, and thus influences the overall malt quality (Dewar, Taylor, & Berjak, 1997; Rimsten, 2003). Germination of barley is a relatively simple and non-chemical approach to improve bioavailability of nutrients (Bamforth & Barclay, 1993; Rimsten, 2003). During the germination process, residual enzymes (amylase, protease, phytase, and β-glucanase) are produced or activated, which may degrade major components such as starch and protein, and/or produce minor nutrients such as gamma-aminobutyric acid (GABA), dietary fiber, and vitamins (Bamforth & Barclay, 1993; Svihus, Newman, & Newman, 1997).

GABA is a four-carbon non-protein amino acid that is produced primarily by the decarboxylation of L-glutamic acid, catalyzed by glutamate decarboxylase (GAD) (Mayer, Cherry, & Rhodes, 1990; Shelp, Bown, & McLean, 1999). Recently, many studies have reported that GABA provides beneficial effects for human health, by

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decreasing blood pressure (Ohmori et al., 1987), helping the recovery from alcohol-related symptoms (Nakagawa & Onota, 1996), controlling stress (Hayakawa et al., 2004), and inhibiting cancer cell proliferation (Oh & Oh, 2004). GABA production in plants is usually enhanced by stress, such as hypoxia, cytosolic acidification, cold shock, mechanical stimulation, water stress and darkness (Oh, 2003; Shelp et al., 1999). Among the plants and cereals, brown rice, beans and green tea have been widely studied in the GABA production (Komatsuzaki et al., 2007). However, only few studies have reported the accumulation of GABA in germinated barley grains (Kihara, Okada, Iimure, & Ito, 2007). Although a number of studies have been carried out to control malting quality under various steeping and germination conditions (MacGregor, 1992), the accumulation of GABA content in waxy hull-less barley has not been reported.

In this study, the effects of various physical conditions for steeping prior to germination and an anaerobic treatment after germination on the accumulation of GABA in germinated waxy hull-less barley grain were investigated.

2. Materials and methods

2.1. Materials

The waxy hull-less barley used in this study was harvested in 2005, and provided by the Honam Agricultural Research Center (Iksan, Korea).

2.2. Steeping and germination

The barley grains were surface-sterilized by dipping in sodium hypochlorite solution (0.1 g/100 g) for 30 min and thoroughly rinsed in deionized water. Steeping was carried out by placing the barley grains (30 g) in a flask containing 100 ml of sterilized deionized water or a buffer solution (pH 6.0, 50 mmol/L sodium acetate) for 8, 16, and 24 h at 5, 15, and 35 °C. The optimal pH for GABA production has been reported to be around 6.0 (Streeter & Thompson, 1972). Generally, steeping is carried out without cooling or heating (Bamforth & Barclay, 1993; Rimsten, 2003), but steeping was done at 5 and 35 °C in this study because temperature stress with cold or heat shocks could enhance the GABA levels (Shelp et al., 1999). After steeping, the barley grains were subjected to germination on two layers of moist filter paper in Petri dish (9 cm) for 24, 48, and 72 h (100% relative humidity) at 15 °C, the temperature recommended for germination of barley (Bamforth & Barclay, 1993; Rimsten, 2003). The germinated barley grains were freeze-dried and ground in a laboratory mill (A10 analytical mill, Tekmar Co., Cincinnati, OH) to a fine powder for analyses.

2.3. Water content during steeping

After steeping, the barley grains were placed on the paper towel to remove surface water and weighted. The moisture content of the grains was determined by drying in a convection oven at 100 °C for 24 h.

2.4. Germination percentage

The barley grains were considered to be germinated when the radicle was 1 mm or longer. The germination percentage was calculated as the number percentage of germinated grains to total grains tested.

2.5. Anaerobic treatment

The effect of anaerobic treatment was evaluated with the barley grains germinated for 48 h at 15 °C after steeping for 8 h at 15 °C in 50 mmol/L sodium acetate buffer solution (pH 6.0). The germinated barley grains (5 g) were placed in a 250 ml flask, and then purged with nitrogen gas for 1 min. After covering tightly, the flask was stored in a dark room at an ambient temperature for 12 h. The grains were then removed from the containers, immediately freeze-dried, and ground by a laboratory mill (A10 analytical mill, Tekmar Co., Cincinnati, OH).

2.6. Analysis of GABA content

The GABA content in the germinated barley grains was determined by following the procedure of Baum et al. (1996) with a minor modification. Ground barley flour (200 mg) was added to a solution (800 μl) of methanol:chloroform:water mixture (12:5:3, volume basis) in a centrifuge tube. The tube was vortexed and then centrifuged at 12,000 × g at 4 °C for 15 min. The supernatant was collected in a flask, and the residue was extracted again with a chloroform:water (3:5, v/v) solution (800 μl). The second supernatant was combined with the first supernatant. The collected sample was dried and then re-dissolved in water. The sample was then filtered through a 0.45 μm filter and the GABA content was measured by an amino acid analysis system (Waters, Milford, MA) after 6-aminoquioly-N-hydroxysuccinimidyl carbonate (AQC) derivatization.

2.7. Statistical analysis

All analyses were performed in triplicate. Statistical analyses were carried out with a Duncan's multiple test ($P < 0.05$) using statistical software SPSS V. 8.2 (SPSS Institute Inc., Cary, NC).

3. Results and discussion

3.1. Water uptake

Fig. 1 represents the water content of the barley grains steeped in water at different temperatures. The grains steeped in the buffer displayed similar results (data not shown). The water content was increased by steeping, and the increment was significant at the early stage of steeping (up to 8 h). It was reported that at the beginning of steeping, the embryo and hull absorbed water more rapidly than did the starchy endosperm (Bamforth & Barclay, 1993).

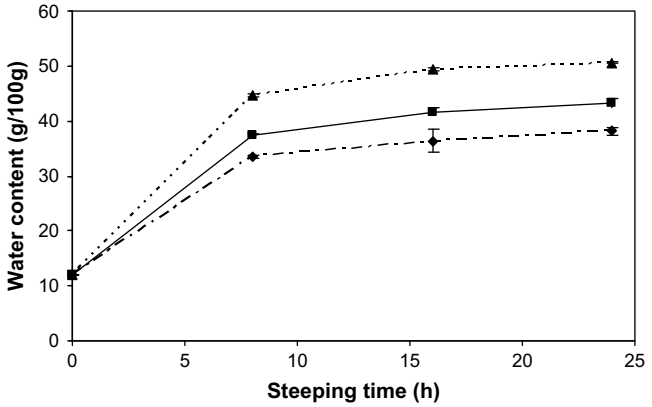

Fig. 1. Moisture content of barley grains steeped in water for 0, 8, 16, and 24 h at 5 (–·◆·–·), 15 (—) and 35 (……) °C. Data reported are the mean ± SD of triplicate determinations.