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Optimizing soaking and germination conditions to improve gamma-aminobutyric acid content in japonica and indica germinated brown rice

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ABSTRACT

Germinated brown rice is a well-known functional food due to its high content of gamma-aminobutyric acid (GABA). This study was designed to test the difference of producing GABA in two domesticated rice genotypes (indica and japonica rice), and the effects of adding exogenous glutamic acid or gibberellin, and processing conditions. Soaking at 30 °C and germination at 35 °C during 36 h resulted in the highest GABA in distilled soaking water with pH 7. The indica rice showed higher GABA levels than japonica rice. GABA was increased under acidic soaking conditions or by adding *L*-glutamic acid (*L*-Glu) at the optimal concentration of 1.0 g L^{-1} and gibberellin A_3 (GA_3) at the optimal concentration of 0.25 mg L^{-1}. The lower accumulation of GABA in japonica rice could be remedied by adding exogenous *L*-Glu and GA_3, and providing acidic soaking conditions. The results help to efficiently produce GABA enriched functional food.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important staple foods of the world, especially in East and South Asia. Brown rice is un-milled and whose outer bran layer is rich in fibre, iron, calcium, vitamins (B1, B2, E, C and D), minerals (Patil & Khan, 2011) and functional compounds such as gamma-aminobutyric acid (GABA), ferulic acid and gamma oryzanol (Matsuo, Sato, Park, Nakamura, & Ohtsuki, 2012). Germinated brown rice (GBR) has higher nutrients levels, sweetness, and better digestion and absorption characteristics than non-germinated brown rice (Wu, Yang, Toure, Jin, & Xu, 2013). GBR could ameliorate cardiovascular diseases risk by modulating lipid metabolism and oxidative stress (Imam et al., 2014). The germinating process of brown rice activates key enzymes, in particular α-amylase,

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β-amylase, β-glucanase, pentosan invertase, maltase, cellulose, protease, nuclease, lipase, phospholipase, and phytase (Charoenthaikij, Jangchud, Jangchud, Prinyawiwatkul, & Tungtrakul, 2010; Ding, Liu, & Zhao, 2011; Li, Cao, & Gu, 2011; Ou, Cheng, & Xing, 2011). Most important of all, GABA is dramatically increased in GBR compared to non-germinated brown rice (Kim, 2013; Roohinejad, Mirhosseini, & Saari, 2009).

GABA is a well-known non-protein amino acid which exists widely in both plants and animals. It is a major inhibitory neurotransmitter in the cerebrospinal fluid of mammals (Chebib & Johnston, 1999; Elliott & Hobbiger, 1959; Liao, Wang, Shyu, Yu, & Ho, 2013). Several health benefits of GABA have been reported (Imam, Azmi, Bhanger, Ismail, & Ismail, 2012), e.g. lower blood pressure (Matsuo et al., 2012) and blood cholesterol (Usuki et al., 2011), greater kidney and liver activity (Kim, Yokozawa, & Nakagawa, 2004), inhibition of cancer cell proliferation (Al-Wadei, Ullah, & Al-Wadei, 2011; Oh & Oh, 2004) and stimulation of cancer cell apoptosis (Abdou, Higashiguchi, & Horie, 2006; Goel, Abbas, & Maiti, 1996). GABA could prevent obesity by ameliorating oxidative stress and high-fat diet-fed disrupted functions of thyroid hormones (Xie, Xia, & Guo, 2014). GABA-enriched foods are therefore seen as functional foods and have become popular to alleviate pain and anxiety, and overcome insomnia and chronic alcohol-related symptoms (Kim, 2013; Vidal-Valverde, Frias, & Sierra, 2002). Foods used to produce GABA are GABA-rich green tea (Huang et al., 2014; Jeng, Chen, Fang, Hou, & Chen, 2007; Sawai, Yamaguchi, Miyama, & Yoshitomi, 2001), germinated wheat (Youn, Park, Jang, & Rhee, 2011), soybean (Aoki, Furuya, Endo, & Fujimoto, 2003; Guo, Chen, & Song, 2011), dairy products (Gobbetti, Di Cagno, & De Angelis, 2010; Inoue et al., 2003; Zbakh & Abbassi, 2012).

In the plant, the GABA biosynthesis pathway is accomplished by GABA shunt and polyamine degradation (Barry, Shelp, Bown, & Michael, 1999). GABA is a metabolic end product and is primarily produced by the decarboxylation of *L*-glutamic acid (*L*-Glu), catalyzed by glutamate decarboxylase (GAD, EC 4.1.1.15) (Bouche & Fromm, 2004). The accumulation of GABA is related to the activity of GAD and substrate concentration of *L*-Glu (Bown & Shelp, 1997). Environmental stresses promote the accumulation of GABA in plant tissue (Kinnersley & Turano, 2000), such as cold or heat stress (Youn et al., 2011), salt stress (Su, Yu, Zhang, & Liu, 2007) and drought stress (Kramer, Breitenstein, Kleinwachter, & Selmar, 2010), mechanical stimulation and oxygen deprivation (Chung, Jang, Cho, & Lim, 2009; Guo et al., 2011) and a lowering of the cellular pH (Bouche, Lacombe, & Fromm, 2003).

Many studies have been conducted on increasing GABA production (Imam et al., 2012), e.g. by xylanase and cellulose enzymes treatments (Das, Gupta, & Kapoor, 2008), bacteria fermentation (Liao et al., 2013; Lu, Chen, & Gu, 2008), or modifying soaking and germination environments of seeds (Komatsuzaki, Tsukahara, & Toyoshima, 2007; Ohtsubo, Suzuki, Yasui, & Kasumi, 2005). The soaking and germination duration, temperature and pH in soaking water have been reported to enhance GABA production in soybean, faba bean and rice (Charoenthaikij et al., 2010; Guo et al., 2011; Li, Bai, & Jin, 2010). Saikusa, Horino, and Mori (1994) reported an optimal temperature of 40 °C for GABA accumulation, while 30 °C was given by Ohtsubo et al. (2005). Thitinunsomboon, Keeratipibul, and Boonsiriwit (2013) recommended combining repeated soaking in tap water at 35 °C for 3 h with incubation at 37 °C for 21 h. The optimal pH for GABA accumulation ranged from 3.0 to 5.8 (Bown & Shelp, 1997; Charoenthaikij, Jangchud, & Jangchud, 2009; Saikusa et al., 1994). A lower cytosolic pH stimulates activity of GAD (Snedden, Chung, Pauls, & Bown, 1992).

Cultivated rice (*O. sativa*) has two subspecies: *O. sativa indica* and *O. sativa japonica* (Huang et al., 2012). Indica rice has long kernels with a length/width ratio of four to five. Basmati and jasmine rice are examples. Japonica rice is the moist, sticky, bright white rice that is used in sushi and Mediterranean and Asian dishes that require stickiness. The length/width ratio is two to three. Roohinejad et al. (2009, 2011) showed differences in the accumulation of GABA among 35 Malaysian rice cultivars. However, information on GABA accumulation in the two main rice subspecies, indica and japonica, is lacking. Glutamic acid rapidly increased GABA accumulation (Liu, Zhai, & Wan, 2005). Gibberellin A_3 (GA_3) increased seed respiration rate and promoted starch degradation along with increased amylase activities, seed germination rate (Li et al., 2013). However, knowledge on the effects of exogenous GA_3, glutamic acid and its interaction with genotypes on GABA yield in GBR is limited.

Though many studies have been done on improving GABA production in GBR, information on the optimal conditions for GABA production in indica and japonica rice is still missing. The objectives of this study were to a) optimize soaking and germination temperature and time for both rice genotypes for GABA production; b) identify optimal pH, *L*-Glu concentration and GA_3 concentration in soaking water for GABA production by indica and japonica rice.

2. Materials and methods

2.1. *Preparation of brown rice and reagents*

Two rice (*O. sativa* L.) cultivars, Jing 305 (*O. sativa japonica*) and Guichao 2 (*O. sativa indica*) (College of Agronomy and Biotechnology, China Agriculture University, Beijing, China) were used in the experiments. The seeds of brown rice were produced using a grain sheller (JLGJ 4.5, Taizhou Meter Co., Zhejiang, China). The seeds were surface-sterilized through dipping in 10 mL L^{-1} sodium hypochlorite solution for 30 m, and then washed with deionized water.

Gamma-aminobutyric acid (GABA) was obtained from Sigma (Sigma-Aldrich Co. LLC, St. Louis, MO, USA). Boric acid, borax, sodium hypochlorite, phenol, disodium hydrogen phosphate, sodium dihydrogen phosphate and absolute ethyl alcohol were obtained locally (Guangdahengyi.Co, Beijing, China) All reagents used were of analytical grade.

2.2. *Experimental design*

Four experiments were performed to study GABA production in two brown rice cultivars, as influenced by environmental conditions (temperature and pH) during soaking and germination, concentration of substrate (*L*-Glu), and concentration of the plant hormone GA_3.