Food Chemistry 271 (2019) 187–192

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Enhanced accumulation of gamma-aminobutyric acid in rice bran using anaerobic incubation with various additives

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ARTICLE INFO

Keywords:
Rice bran
Gamma-aminobutyric acid
Anaerobic incubation
Hydrolyzed wheat protein
Yeast extract
Pyridoxal-5-phosphate

ABSTRACT

An anaerobic incubation for the enzymatic production of gamma-aminobutyric acid (GABA) in rice bran with the addition of glutamate, hydrolyzed wheat protein (HWP), yeast extract (YE) and pyridoxal-5-phosphate (PLP) was investigated. Rice bran was moistened (30% moisture content) with an electrolyzed oxidizing water and anaerobically incubated under nitrogen at 40 °C for 8 h. The incubation activated the glutamate decarboxylase (GAD) in rice bran and increased the GABA content from 10.7 to 171.5 mg/100 g. The addition of glutamate and protein hydrolysates further amplified the GABA content in the treated rice bran: 974.9, 487.4, and 372.8 mg/100 g, with 2.25% glutamate, 6% HWP, and 8% YE, respectively. Furthermore, addition of PLP (1.48 mg/100 g) as a coenzyme for GAD, together with 2.25% glutamate addition, could raise the GABA accumulation in rice bran to 2242 mg/100 g. Other amino acids in rice bran were changed in their composition by the anaerobic treatment.

1. Introduction

Rice bran, a by-product from milling of brown rice to prepare white rice, comprises 5–8% of the total rice grain. It includes the pericarp, seed coat, aleurone layer, embryo, and outer portion of starchy endosperm (Friedman, 2013; Wang, Suo, de Wit, Boom, & Schutyser, 2016). Rice bran contains numerous nutrients including fiber, minerals, and vitamins (Patil & Khan, 2011), as well as various health-promoting phytochemicals such as gamma-oryzanol, gamma-aminobutyric acid (GABA), phenolic acids, flavonoids, tocopherols, and phytic acid (Bhat & Riar, 2017; Friedman, 2013). Among the phytochemicals, GABA has been extensively studied because of its unique and potent functions. It is a non-protein four-carbon amino acid found in diverse biomaterials in microorganisms, plant and animal tissues (Diana, Quílez, & Rafecas, 2014), functioning as a chief inhibitory neurotransmitter in the central nervous system of mammals (Bouché, Lacombe, & Fromm, 2003). In addition, GABA exhibits other physiological functions beneficial to human health. For instance, it exhibits anti-stress effect (Vaiva et al., 2004), improves learning and memory ability (Kalueff & Nutt, 1996), reduces blood pressure (Inoue et al., 2003), and acts even as an anti-cancer agent (Oh & Oh, 2004). At present, many processed foods enriched with GABA are commercially available worldwide, even for the regulation of insomnia and symptoms related to chronic alcoholism (Garcia & Salloum, 2015; Lai, Chen, Chen, Chang, & Cheng, 2012).

In general, the synthesis of GABA in plants involves the combination of GABA shunt and polyamine degradation. It is metabolically produced from the decarboxylation of glutamate catalyzed by the enzyme glutamate decarboxylase (GAD) (Bouché et al., 2003), and the degradation of polyamine catalyzed by diamine oxidase and polyamine oxidase (Oh & Oh, 2004). The GAD could be activated by a coenzyme, pyridoxal-5-phosphate (PLP), leading to an increase in the production of GABA. Therefore, the enzymatic synthesis of GABA in plants may be improved by providing glutamate and activating GAD (Zhang et al., 2014). A substantial increase in GABA content in rice germ by 15 times was observed by the addition of glutamate and PLP (Ohtsubo, Asano, Sato, & Matsumoto, 2000).

Protein hydrolysates are widely utilized in foods as functional or nutritional ingredients because of the richness in amino acids and peptides (Wu, Baek, Gerard, & Cadwallader, 2000). Among those, hydrolyzed wheat protein (HWP) contains a large content of glutamate from the conversion of glutamine by enzymatic deamination (Hamada, 1992). Yeast extract (YE) could be another protein hydrolysate obtained from the autolysis of baker's yeast (*Saccharomyces cerevisiae*), which is widely used as a food additive for the preparation of soups, gravies, sauces and snacks (Nagodawithana, 1992; Tanguler & Erten, 2008).

Environmental stress such as water addition, anaerobic storage, temperature fluctuation, and lack of light may be used to accelerate the

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https://doi.org/10.1016/j.foodchem.2018.07.175
Received 6 March 2018; Received in revised form 15 June 2018; Accepted 25 July 2018
Available online 26 July 2018

GABA synthesis in plant tissues (Kinnersley & Turano, 2000; Poojary et al., 2017). In general, the stressful conditions could increase the cytosolic Ca^{2+}, calmodulin, or H^+ levels, leading to the reduction in the cytosolic pH and subsequent increase in GAD activity (Komatsuzaki et al., 2007). Watchraparpaiboon, Laohakunjit, Kerdchoechuen, and Photchanachai (2007) reported that soaking in an acidic water (pH 6.0) at 35 °C for 24 h improved the production of GABA in brown rice. The residual content of GABA in tea leaves was increased by an anaerobic storage for 6 h (Tsushida & Murai, 1987). In addition, multi-stress treatments using two or more treatments were found to be more effective in the accumulation of GABA in plants than the single treatments. Komatsuzaki et al. (2007) reported that a combination of soaking and gaseous treatment significantly improved the production of GABA in rice during germination.

Most of the studies for GABA production in rice have been carried out by fermentation or germination. Major drawbacks in those processes include requirement of extensive period and production of unpleasant odor from the metabolic process or microbial contamination. Limited research is carried out in increasing the GABA production in rice bran using multi-stress treatments, which may shorten the processing time and minimize the formation of undesirable odor. Although the addition of glutamate and/or chitosan has been performed to raise the GABA production in rice bran, no study has been carried out in the GABA accumulation in rice bran using environmental stress with additives. In the present study, an effective process was developed to boost up the accumulation of GABA in rice bran using an anaerobic incubation with the addition of glutamate, protein hydrolysates such as HWP and YE, and PLP as a coenzyme for GAD.

2. Materials and methods

2.1. Materials

The rice bran was obtained from a milling factory of brown rice in CJ CheilJedang Co., (Busan, Korea). The brown rice (*Oryza sativa* subsp. *Japonica*) was polished at 10% degree of milling, and the rice bran obtained (~10% moisture content) was stored in a deep freezer (−60 °C) prior to use. The hydrolyzed wheat protein (HWP) and yeast extract (YE) used in this study were obtained from Meail Food Co. (Seoul, Korea), and BD Difco Co. (Detroit, USA), respectively. Electrolyzed oxidizing water (EOW, pH 2.8) was prepared using a water electrolyzer (BTM-207D, Bion-tech, Gunpo, Korea).

The GABA, PLP, glutamate, amino acid standards, sulfosalicylic acid, borax, sodium azide, and derivatization reagents including o-phthaldialdehyde (OPA), 3-mercaptopropionic acid (MPA), and 9-fluorenylmethoxycarbonyl chloride (FMOC) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). High-performance liquid chromatography (HPLC)-grade acetonitrile, ethanol, methanol, and acetic acid were supplied by Thermo Fisher Scientific Inc. (Waltham, MA, USA). All other chemicals and reagents used were of analytical grade.

2.2. Analysis of GABA and glutamate

The contents of GABA and glutamate in rice bran samples were determined according to the procedure described by Kim, Lee, Lim, and Han (2015). Rice bran samples (1.0 g, dry basis) were dispersed in a mixture of 4% sulfosalicylic acid solution (4.5 mL) and 70% ethanol (4.5 mL). After shaking for 30 min in a water bath at room temperature, the suspension was centrifuged at 3000 × *g* and 4 °C for 15 min and the supernatant was collected. This extraction process was performed three times and the collected supernatant was filtered through a polyvinylidene fluoride filter (0.20 μm pore size, Advantec, Tokyo, Japan) for HPLC analysis

The content of GABA and glutamate were analyzed according to the HPLC method described by Herbert, Santos, and Alves (2001). The analysis was performed using an HPLC system (Dionex UltiMate 3000, Thermo Fisher Scientific Co., Ltd., MA, USA), with pre-column derivatization using 0.1 M borate buffer containing OPA and MPA. The HPLC system was equipped with a gradient pump (LPG-3400SD), an automated sample injector (WPS-3000SL), a fluorescence detector (FLD, UltiMate 3000, Thermo Fisher Scientific Co., Ltd., MA, USA), and a C18 column (6 mm × 150 mm, Acclaim 120, Dionex, Sunnyvale, CA, USA). Mobile phases A (10 mM borax, 10 mM sodium phosphate, and 0.5 mM sodium azide, pH 8.2) and B (45% acetonitrile, 45% methanol, and 10% distilled water) were used under a multistep gradient program as follows: 0–12 min, 10% B; 12–13 min, 10–30% B; 13–17 min, 30–90% B; and 17–20 min, 90–100% B. The flow rate was 1.2 mL/min at 40 °C, and the sample was measured under an excitation wavelength of 335 nm and fluorescence wavelength of 390 nm.

2.3. Anaerobic incubation

Rice bran samples (100 g, wet basis) were moistened by spraying EOW (~20 mL) to adjust the moisture content to 30.0 ± 1.0% and placed in a plastic bag (Supplementary Table 1). For anaerobic treatment, the rice bran was purged with nitrogen gas for 2 min and the sealed plastic bag was stored in a dark place at 40 °C for up to 12 h. During the anaerobic incubation, GABA content in rice bran was analyzed at different time intervals.

2.4. Determination of pH

The pH value of native and treated rice bran samples (1.0 g/9.0 mL distilled water) was determined using a digital pH meter calibrated at room temperature. All measurements were performed in triplicates.

2.5. Addition of glutamate, HWP, and YE

The effect of different additives including glutamate, HWP, and YE on the GABA accumulation in the anaerobically treated rice bran was analyzed using the following procedure. The EOW solutions containing different concentrations of glutamate (100, 200, 400, 600, 800, and 1000 mM) were prepared and used to moisten the rice bran (30% moisture). Considering that approximately 20 mL of the EOW was consumed, the concentrations of glutamate in rice bran became approximately 0.37, 0.75, 1.50, 2.25, 2.99, and 3.74 g/100 g (wet basis), respectively for the EOW solutions containing 100, 200, 400, 600, 800, and 1000 mM glutamate. The HWP and YE were added to rice bran following the same procedure by dissolving those in EOW. The amount of protein hydrolysates was controlled to make the final concentration to be 2–10% (wet basis). The rice bran samples containing different additives including glutamate, HWP, and YE were subjected to the anaerobic incubation at 40 °C for 8 h.

2.6. Addition of pyridoxal-5-phosphate

Different concentrations of PLP (0.49–4.95 mg/100 g, wet basis) was added to rice bran samples (30% moisture) containing 2.25% glutamate, 6% HWP, or 8% YE, and then the rice bran samples were subjected to the anaerobic incubation at 40 °C for 8 h.

2.7. Determination of amino acid composition

The rice bran extracts in sulfosalicylic acid/ethanol solution, which were used for GABA analysis, were also used to evaluate the overall amino acid composition. The changes in the amino acid composition of rice bran was analyzed according to the HPLC method described by Herbert et al. (2001). The amino acids in rice bran extracts were analyzed by a fluorescence detector at excitation wavelength of 340 nm and fluorescence wavelength of 450 nm.

The mobile phases A (10 mM borax, 10 mM sodium phosphate, and 0.5 mM sodium azide, pH 9.0) and B (45% acetonitrile, 45% methanol,