Effect of Air-Phase Germination with Anion Radiation and Water-Spraying on Germination Ratio, Sprout Growth, and GABA Contents of Germinated Brown Rice

K. T. Lim, J. H. Chung, J. H. Hong, J. H. Kim, E. T. Lee, A. L. Im

Abstract: The objective of this study was to investigate the effects of air-phase germination with water-spraying and anion stimuli on germination ratio, sprout growth and γ-aminobutyric acid (GABA) of brown rice. Air-phase germination method with intermittent spraying water improved germination ratio and sprout growth by about 100% compared with the conventional water-soaking method. Anion radiation was applied during the germination process and improved the germination ratio, sprout growth and color quality of the germinated brown rice. Germination ratio and sprout growth were improved up to 9% with anion radiation, and its brightness was higher than brown rice germinated with no anion radiation. The air-phase germination with water-spraying improved the GABA content of germinated brown rice by about 8~9 times compared with that of brown rice.

Keywords: Air-phase Germination, Water-spraying, Anion Stimuli, Germination Ratio, Sprout Growth

Introduction

Important factors known for rice germination include oxygen, water, and temperature. Germinated brown rice contains much more fiber than conventional brown rice, up to three times the amount of lysine, an essential amino acid, ten times the amount of γ-aminobutyric acid (GABA), and an additional amino acid known to improve kidney function. Also, brown rice sprouts contain a potent inhibitor of an enzyme called protylendopetidase, which has been implicated in Alzheimer's disease (Hiroshi, 2000). A common method for germinating brown rice is soaking in water, a slow process controlled by the diffusion of water in the grain. Warm water-soaking is used to shorten the soaking time since high temperature increases the hydration ratio (Engles *et al.*, 1986). However, soaking in water may cause microbial contamination, which affects the color, taste and smell of the product. A second germination method using air-phase reduces germination time but decreases rice quality due to short pollination and air pollution. Therefore, an alternative method was required to accelerate the germination of brown rice and to improve the quality of germinated rice.

There are internal factors that retard germination such as embryo-structure and chemical causation in addition to external factors such as pollination, temperature, oxygen, and light (Adams, 1999). Kum *et al.* (2004) investigated the nutritional composition and physiochemical properties of germinated brown rice. The properties of germinated brown rice were affected by color and flavor. Velupillai and Verna (1982) proposed the use of hydrostatic pressure during soaking to accelerate germination of seeds. The growth of microorganisms and the differentiation of plant callus may be increased when cells are exposed to low intensity currents. The effect of mechanical stimulation in high temperature tomato seedlings quality and growth was investigated by Choi *et al.* (2001). They reported that proper application of these treatments could control excessive overgrowth without reducing yield. Also, an increase of germination ratio in Welsh onion and spinach seeds with a low-dose γ-ray treatment was reported by Kim *et al.* (2000). Besides germination of seeds, methods to increase GABA contents have been studied recently. GABA has several physiological functions such as neurotransmission and induction of hypotensive effects, diuretic effects, and tranquilizing effects (Jakobs *et al.*, 1983). To develop a processing method for high concentration of GABA, the effects of the soaking and sprout processing method on GABA content in brown rice were studied (Komatsuzaki *et al.*, 2003; Noriko *et al.*, 1999). Kinefuchi *et al.* (1999)

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reported that GABA was higher in brown rice processed by a high-pressure treatment. Jung *et al.* (2004) reported that treatment with chitosan and glutamic acid improved the levels of GABA during the brown rice germination. Suzuki *et al.* (1999) reported that the sprout growth of brown rice under air-phase was more rapid than liquid phase. The improvements required as a new technique are an increase in the production and a reduction in costs. Expertise deficiencies of post-harvesting process could reduce the product quality of brown rice (Kwon *et al.*, 1995; Lee *et al.*, 2000).

The objective of this study was to investigate the effects of air-phase germination with water-spraying and anion radiation on the germination ratio, sprout growth and GABA content of brown rice.

Materials and Methods

1. Materials

The brown rice (Dongjin variety) used in this study was harvested at Jangsung of Chonnam Province in 2005. It was packed in polypropylene bags and stored at 4℃ in a refrigerator. Prior to the experiment, the brown rice samples were cleaned thoroughly and defective grains were removed. Only sound grains were used for the experiment. The initial moisture content was determined by a moisture determination balance (FD600, Kett Electric Laboratory, Tokyo, Japan) at 100℃ for 60 min after crushing with a miller (IKA-WERKE A10, Japan). The initial moisture content, based on 3 replications, was 14.3% (wet basis). Linear dimensions and weight of grains were measured with a digital caliper having a resolution of 0.01 mm (CD-15CD, Mitutoyo Co., Japan) and a precision electronic balance (TE 214S, Sartorius, Japan) having a resolution of 0.01 mg-1 g, respectively. Fifty kernels of brown rice with germs were selected for each test of the water-soaking method. The germination tests using the soaking method were conducted according to the criteria of Association of Official Seed Analysis (1993) : brown rice was soaked in a constant temperature and humidity apparatus (Dasol Scientific Co., Korea) at 25℃ and 90% humidity for 48 h after soaking at 25℃ for 6 h. The water-soaking method was used as control in this study. Experiments using the water-soaking method were conducted in Petri dishes (9.0 cmx1.5 cm) with filter paper (No. 2, Whatman, Kent, England). The sprout length was measured using a digital caliper every 12 h. The sprout length of germinated brown rice in each treatment was calculated by measuring and averaging the sprout lengths of 50 kernels.

The germinating experiments were conducted with 20 min intervals of water-spraying at 25℃ and 90% relative humidity. Anions were radiated using an anion lamp apparatus (EFTR20EX-D, Samjung Co., Korea). The number of anions was measured with an ion digital device (ITC -201A, INTI Co., Japan).

2. Experiments with air-phase germination using water-spraying and anions

Experiments of air-phase germination with water-spraying and anions were performed as shown in Fig. 1. Experiments were conducted with samples of 150 g (depth : 2 mm) and 1000 g (depth : 10 mm) in tray baskets, respectively. The brown rice samples were pre-washed in water at 25℃. Tray baskets were placed on the overhead rack of chamber. Water spraying was operated with a water pump and spraying nozzles by a controller programmed for a 20 min cycle. Germination experiments were performed at 25℃ and 90% relative humidity for 48 h with anion radiation after soaking at 25℃ for 6 h. Germination ratio and sprout length of brown rice were monitored at an interval of 12 h up to 48 h, respectively. After stopping the germination process with the controller, brown rice was dried at 40℃ for 24 h, and the germinated brown rice was removed.

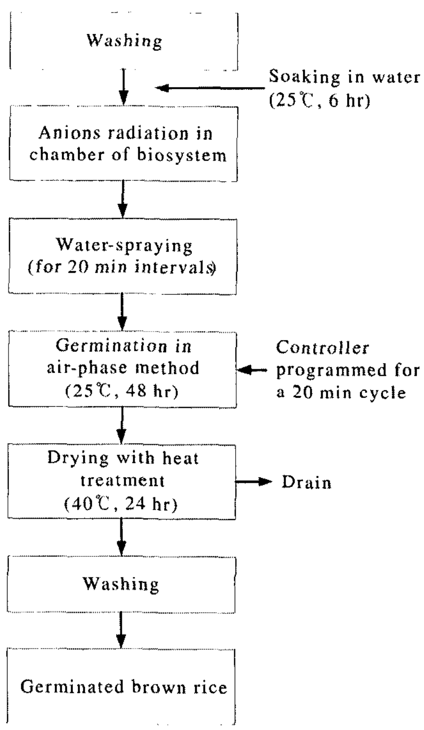

Fig. 1 Germinated brown rice process of an air-phase germination method with anion radiation and water-spraying.

3. Analysis of GABA content of brown rice

The GABA content was analyzed according to the method of Oh *et al.* (2004) and Park *et al.* (2005). 800 ul mixed organic solvent solution (methanol : chloroform: water = 12:5:3) was added to 200 mg freeze-dried sample. The aqueous solution layer containing GABA was obtained through micro centrifugation (15,000 rpm, 4℃, 15 min), and the obtained supernatant was re-centrifuged to remove the remnant impurities. The supernatant was then freeze-dried, suspended in water, filtered through a 0.45 um PVDF (polyvinlidene difluoride) membrane, and analyzed. AccQ Tag Reagent was used to implement the fluorescence derivation of GABA. To separate the derivatives, 3.9x150 mm AccQ Tag^{TM} (Nova-Pak^{TM} C18, Waters) column was used. The GABA content was calculated using a commercial GABA standard based on a standard curve, expressed in mg/g.

4. Color measurements of brown rice germinated

The effect of the anion radiation on the color quality of germinated brown rice was investigated. The color of germinated brown rice was measured by a color difference meter (Minolta chromameter CR-300, Japan) under D_{65} light source. Five measurements were taken for each sample. Before measuring, the color difference meter was standardized with a white calibration plate (Calibration Plate CR-A43 for the CR-300) provided with the instruments. The tristimulus values X, Y, and Z of all specimens were obtained directly from the color difference meter. From the data of L* (value on the white/black axis), a* (value on the red/green axis), and b* (value on the blue/yellow axis) color parameters, the color difference, $\triangle E^*$, ($\triangle E^*=[(\triangle L^*)^2+(\triangle a^*)^2+(\triangle b^*)^2]^{1/2}$) was calculated as established by the Commission Internationale de l'Eclairage (CIE) in 1976.

5. Statistical analysis

The statistical analysis was carried out using the SAS Statistical Analysis System for Windows v8.2 (SAS Institute, Inc., Cary, NC, USA). Statistical significance between control and treatment groups was compared with two-way ANOVA test at $p < 0.05$. The data are reported as mean ± standard deviation (SD).

Results and Discussion

1. Effect of air-phase germination with water-spraying

A comparison of germination ratio between the air-phase germination with water-spraying with samples of 1000 g (depth : 10 mm) without anion in tray baskets and the water-soaking method (control) is shown in Fig. 2. The air-phase germination with water-spraying without anion in this study improved the germination ratio of brown rice by about 100%, compared with the water-soaking method. The difference between soaking and air-phase method in Fig. 2 was found to be statistically significant ($p < 0.05$). Fig. 3 shows the comparison of sprout growth between the air-phase germination with water-spraying with samples of 1000 g (depth : 10 mm) without anion in tray baskets and the water-soaking method. The air-phase germination with water-spraying method improved the sprout growth by about 70%, compared with the water-soaking method. The difference between soaking and air-phase method in Fig. 3 was found to be statistically significant ($p < 0.05$).

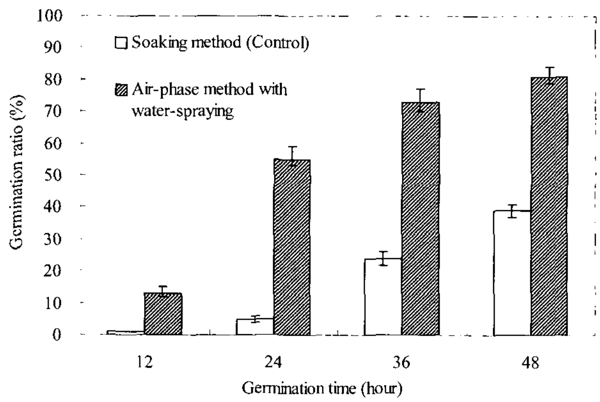

Fig. 2 Comparison of germination ratio between the air-phase germination method with water-spraying without anions for 1000 g samples (depth : 10 mm) and the water-soaking method (control).

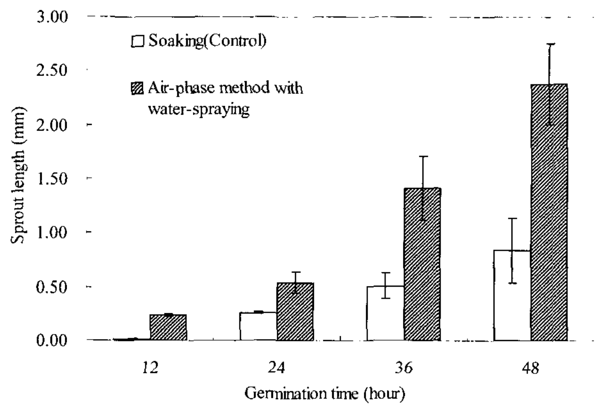

Fig. 3 Comparison of sprout growth between the air-phase germination method with water-spraying without anions for 1000 g samples (depth : 10 mm) in tray baskets and the water-soaking method (control).

2. Effect of air-phase germination with water-spraying and anion radiation

Fig. 4 and Fig. 5 show the comparison of germination ratio between the air-phase germination with anions and the air-phase germination without anion radiation for samples of 150 g (depth : 2 mm) and 1000 g (depth : 10 mm) in tray baskets, respectively. Significant level of the effects of 24, 36, and 48 h treatments on the germination ratio in Fig. 4 was 5% ($p < 0.05$), and that of 12 h treatment was 1% ($p < 0.1$). And, Significant level of 24, 36 h treatments on the germination ratio in Fig. 5 was 5% ($p < 0.05$), but 48 h treatment was less significant ($p < 0.1$).

In experiments of the anions radiation, the effect of anions on the germination ratio of brown rice in a single layer of the 150 g sample was about 9% higher than that of the 1000 g sample in tray baskets. However, the anions treatment for the 1000 g sample improved the germination ratio of brown rice by only 4% compared with that of sample weights of 1000 g without anions, due to the multiple layers of brown rice in each basket.

Fig. 6 and Fig. 7 show the comparison of sprout growth between the air-phase germination with anion and the air-phase germination without anion radiation for samples of 150 g and 1000 g in tray baskets, respectively. Significant level of the effects of 36 and 48 h treatments on the sprout growth in Fig. 6 was significant ($p < 0.05$), whereas 12 and 24 h treatment was significant ($p < 0.1$). The effects of each treatments on the sprout growth in Fig. 7 were no significant. The air-phase germination with anion radiation in this study improved the sprout growth of

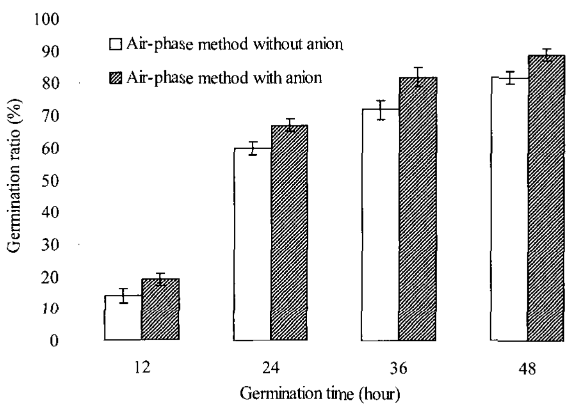

Fig. 4 Comparison of germination ratio between the air-phase germination method with anion radiation and the air-phase germination without anions for 150 g samples (depth : 2 mm) in tray baskets.

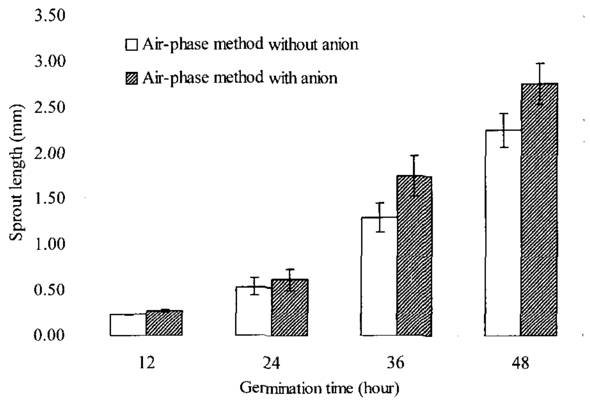

Fig. 6 Comparison of sprout growth between the air-phase germination method with anion radiation and the air-phase germination without anions for 150 g samples (depth : 2 mm) in tray baskets.

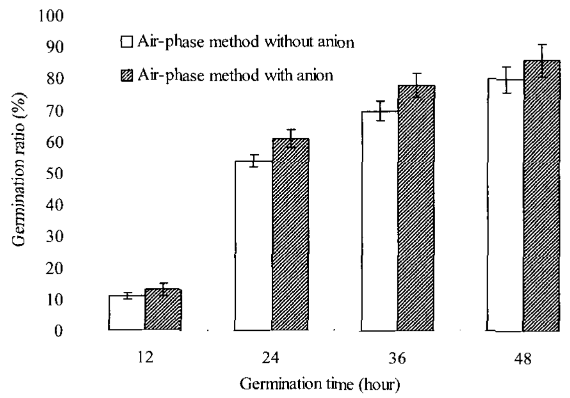

Fig. 5 Comparison of germination ratio between the air-phase germination method with anion radiation and the air-phase germination without anions for 1000 g samples (depth : 10 mm) in tray baskets.

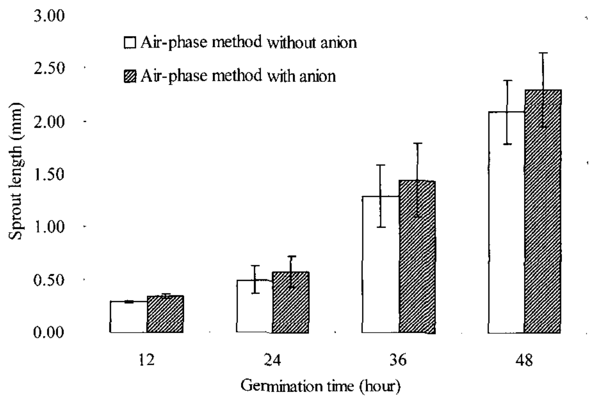

Fig. 7 Comparison of sprout growth between the air-phase germination method with anion radiation and the air-phase germination without anions for 1000 g samples (depth : 10 mm) in tray baskets.

brown rice in a single layer (depth : 2 mm) of the 150 g sample by about 30%, compared with that of the 1000 g sample weights (depth : 10 mm) without anion radiation. However, the anion radiation in the 1000 g sample improved the sprout growth of brown rice by only 8% compared with that of the 1000 g samples without anions. Obviously the anion radiation affected only the a single layer of samples in the baskets.

3. Color quality of brown rice germinated with the anion radiation

The effect of the anion radiation on the color quality of germinated brown rice was investigated. Table 1 shows the comparison of color quality of germinated brown rice between the air-phase germinated without anions and the air-phase germination method with anions radiation, compared with that of soaking method (control). The water-soaking method (A) showed the significant effect on the color of the brown rice germinated at 25℃ and 90% humidity for 48 h after soaking at 25℃ for 6 h. However, the air-phase germination method with water-spraying with anion (B) showed higher value in brightness than the air-phase germination method without anion radiation (D). The values of $\triangle E$ obtained were present 32.66±1.91 (B) and 32.66±1.91 (D) compared with 38.36±1.37 (A), which were significantly different respectively (p < 0.05). Also, this result indicates that the air-phase germination method with anions in a single layer (B) had higher values in brightness than air-phase germination with anion radiation in sample in bulk (C). According to these results, brown rice germinated with anions showed higher brightness than brown rice germinated without the anion radiation.

Table 1 Comparison of color quality of germinated brown rice between the air-phase germination method with and without anions and the water-soaking method (control)

Treat-ments	Color			
	L*	a*	b*	ΔE*
A	79.56±1.94	2.88±0.70	23.02±2.57	27.61±2.76
B	74.66±0.98	3.09±0.82	25.42±1.59	32.66±1.91
C	71.52±1.18	3.37±0.90	24.13±1.15	34.06±1.13
D	65.71±1.74	3.14±0.45	23.69±0.74	38.36±1.37

A : Water-soaking method (control)
B : Air-phase germination method with anion radiation for a single layer for the 150 g sample (depth : 2 mm) in a tray basket
C : Air-phase germination method with anion radiation for the 1000 g sample (depth : 10 mm) in bulk
D : Air-phase germination method without anion radiation

4. GABA contents of brown rice germinated

GABA contents of germinated brown rice are shown in Table 2. The brown rice germinated by the air-phase method with water-spraying had GABA content approximately 8-9 times higher than that of the control. Also, the GABA content in brown rice germinated by the air-phase method with water-spraying was similar to that of brown rice germinated using the water-soaking method.

Table 2 GABA contents of raw brown rice (control), rice germinated by the water-soaking method, and rice germinated by the air-phase method with water-spraying

Samples	GABA contents	Remark
A	36.49±0.01 mg/100 g	2 replication
B	40.78±5.2 mg/100 g	2 replication
C	39.48±4.3 mg/100 g	2 replication
D	43.33±5.5 mg/100 g	2 replication
F	4.7 mg/100 g	2 replication

A : Brown rice germinated by an air-phase method with *water-spraying*
B : Brown rice germinated by a water-soaking method
C : Glutinous brown rice germinated by a water-soaking method
D : Black brown rice germinated by a water-soaking method
F : Normal brown rice (control)

Conclusions

In this study, a brown rice germinating biosystem of air-exposure type was developed, which could germinate the brown rice within 48 h without water-soaking. Also, germinating technology using anion radiation improved the color quality of the germinated brown rice. Air-phase germination method with intermittent spraying water improved germination ratio and sprout growth by about 100%, compared with the conventional water-soaking method. Anion radiation was applied during the germination process and improved the germination ratio, sprout growth and color quality of the germinated brown rice. Germination ratio and sprout growth were improved up to 9% with anion radiation, and brightness was higher than brown rice germinated with no anion radiation. The air-phase germination with water-spraying improved the GABA content of germinated brown rice by about 8~9 times compared with that of brown rice.

Acknowledgments

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References

Hiroshi Kayahara. 2000. Soaked brown rice is better for you, News in Science, Available from : http //www.abc.net.au/science/news/stories/s225249. Accessed Dec. 19.

Engles, C, M. Hendrickx., S. De Samtlas., I. De Gryze and P. Tobback. 1986. Modelling water diffusion during long-grain rice soaking. J. Food Eng. 5:55-73.

Adams, R. 1999. Germination of Callitris seeds in relation to temperature water stress, priming and hydration-dehydration cycles. J. Arid Environ. 43:437-448.

Kum, J. S., B. K. Chio., H. Y. Lee., J. D. Park and H. J. Park. 2004. Physicochemical properties of germinated brown rice. Korea J. Food Preserv. 11(2):182-188.

Velupillai, L and L. R. Verna. 1982. Parboiled rice quality as affected by the level and distribution of moisture after the soaking process. Tran. of the ASAE. 25:1450-1456.

Choi, Y. H., H. C. Rhee., D. K. Park., J. K. Kwon and J. H. Lee. 2001. Effects of mechanical stimulation and chemical treatments on growth of seedling and yield of tomato. Kor. J. Hort. Sci. & Technol. 19(3):320-324.

Kim, J. S., E. K. Lee., M. H. Back., D. H. Kim and Y. B. Lee. 2000. Influence of low dose γ radiation on the physiology of germinative seed of vegetable crops. Kor. J. Environ. Agric. 19(1):58-61.

Jakobs, C., J. Jaeken and KM. Gibson. 1993. Inherited disorders of GABA metabolism. J. Inherited Metabolic Disease. 16(4):704-715.

Komatsuzaki., K. Tsukahara., H. Toyoshima., T. Szuki., N. Shimizu and T. Kimura. 2003. Effect of soaking and gaseous phase sprout processing on the GABA content of pre-germinated brown rice. ASAE paper No. 036073.

Noriko Komatsuzaki., Kikuichi Tsukahara., Hidechika Toyoshima., Tadanao Suzuki., Naoto Shimizu and Toshinori Kimura. 2007. Effect of soaking and gaseous treatment on GABA content in germinated brown rice. J. Food Eng. 78(2):556-560.

Kinefuchi, M., M. Sekiya., A. Yamazaki and K. Yamamoto. 1999. Accumulation of GABA in brown rice by high pressure treatment. Nippon Shokuhin Kagaku Kougaku Kaishi. 46(5):323-328.

Jung, G. H., N. Y. Park., S. M. Jang., J. B. Lee and Y. J. Jeong. 2004. Effects of germination in brown rice by addition chitosan/glutamic acid. Korea J. Food Preserv. 11(4):538-543.

Suzuki, K and T. Maekawa. 1999. Analysis of an sprouting control of brown rice, J. of Soc. of Agri. Struc. 30(1):1-10.

Kwon, B. S., E. T. Lee., D. H. Chung., H. J. Park and S. R. Lee. 1995. The effects of the planting time of mother bulb and mulching materials for the seed production of onion. J. Oriental Bot. Res. 8:247-252.

Lee, S. C and S. W. Park. 2000. Characteristics of pellet seed on germination and emergence in onion. Kor. J. Plant Res. 13:41-47.

Association of official seed analysis (AOSA). 1993. Rules for testing seeds. J. Seed Technol. 16:1-13.

Oh, C. H and S. H. Oh. 2004. Effect of germinated brown rice extract with enhanced levels of GABA on cancer cell proliferation and apoptosis. J. Med. Food. 7:19-23.

Park, K. B and S. H. Oh. 2005. Production and characterization of GABA rice yogurt. Food sci. Biotechnol. 14(4):518-522.