JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 9759–9763 9759
DOI:10.1021/jf101268a

Dynamic Changes in γ-Aminobutyric Acid and Glutamate Decarboxylase Activity in Oats (*Avena nuda* L.) during Steeping and Germination

Jian Guo Xu,[†] Qing Ping Hu,*[,‡] Jiang Lian Duan,[†] and Cheng Rui Tian[§]

[†]College of Engineering, Shanxi Normal University, 1 Gongyuan Street, Linfen City, China 041004, [‡]College of Life Sciences, Shanxi Normal University, 1 Gongyuan Street, Linfen City, China 041004, and [§]College of Food Engineering and Nutritional Science, Shaanxi Normal University, 199 South Chang'an Road, Xi'an City, China 710062

γ-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the central nervous system and provides beneficial effects for human and other animals health. To accumulate GABA, samples from two different naked oat cultivars, Baiyan II and Bayou I, were steeped and germinated in an incubator. The content of GABA and glutamic acid as well as the activity of the glutamate decarboxylase (GAD) in oats during steeping and germination were investigated with an amino acid automatic analyzer. Compared with raw groats, an increase in GABA content of oat groats during steeping and germination was continuously observed for two oat cultivars. The activity of GAD increased greatly at the end of steeping and the second stage of germination for Baiyan II and Bayou I, respectively. Glutamic acid content of treated oat groats was significantly lower than that in raw groats until the later period of germination. GABA was correlated ($p < 0.01$) significantly and positively with the glutamic acid rather than GAD activity in the current study. The results indicates that steeping and germination process under highly controlled conditions can effectively accumulate the GABA in oat groats for Baiyan II and Bayou I, which would greatly facilitate production of nutraceuticals or food ingredients that enable consumers to gain greater access to the health benefits of oats. However, more assays need to be further performed with more oat cultivars.

KEYWORDS: γ-Aminobutyric acid; glutamate decarboxylase activity; oats (*Avena nuda* L.); steeping; germination; malting

INTRODUCTION

γ-Aminobutyric acid (GABA) is a nonprotein amino acid that is widely found in many plants, animals, and microorganisms (*1*). It was reported that GABA is the principal inhibitory neurotransmitter in the central nervous system in animals (*2*) and provides beneficial effects for human and other animals' health by decreasing blood pressure (*3*), preventing chronic alcohol-related diseases (*4*), and inhibiting cancer cell proliferation (*5*). Therefore, accumulation of GABA in the plants and some cereals, including brown rice (*6*), tea (*7*), wheat bran (*8*), and soybean (*9*), has been studied. Many studies reported that GABA is a metabolic end-product and is primarily produced by the decarboxylation of L-glutamic acid, catalyzed by glutamate decarboxylase (GAD, EC 4.1.1.15) (*10*). In plants, the GABA pathway is mainly composed of three enzymes: (1) a pathway catalyzed by cytosolic enzyme glutamate decarboxylase (GAD) that leads to forming GABA by direct and irreversible decarboxylation of L-glutamic acid, (2) mitochondrial enzymes of GABA transaminase (GABA-T, EC 2.6.1.19) that catalyze the reversible conversion of GABA to succinic semialdehyde using either pyruvate or α-ketoglutarate as amino acceptors, (3) a pathway catalyzed by succinic semialdehyde dehydrogenase (SSADH, EC 1.2.1.16) that irreversibly oxidizes succinic semialdehyde to succinate (*1*, *10*). Among the three enzymes, the catalyzed reaction by GAD is considered as the rate limiting step in a GABA pathway (*1*, *10*). Recently, GABA has been accumulated by stimulating the activity of GAD with various environmental stress, such as anoxia (*11*), water stress, as well as decreasing cellular pH, temperature changes, and mechanical stress (*12*).

Cereal seeds have been malted for centuries to improve bioavailability of nutrients (*13*), and during the malting process including steeping and germination, endogenous enzymes are produced or activated, which may degrade major components such as starch and protein, and/or produce some secondary metabolites such as GABA and phenolics (*14*, *15*). Stress from steeping and germination is also reported to be effective for stimulating the production of GABA in some cereal seeds such as brown rice (*14*), foxtail millet (*16*), barley (*11*), and buckwheat (*17*) and so on. Therefore, we speculate that steeping and germination may have a positive impact on the accumulation of GABA in oat groats, which has not been reported as yet.

In our previous study, we have reported changes in phenolic compounds and activity in oats during germination (*15*). As a

*To whom correspondence should be addressed. Phone: +86-357-2051247. Fax: +86-357-2051000. E-mail: xjg71@163.com).

Published on Web 08/09/2010

follow-up to a joint study, the objective of the present study was to investigate the effect of a highly controlled steeping and germination process on the content of GABA, as well as activity of GAD and its substrate glutamic acid in oat groats. This was performed to gain a more collective and comparative picture of what happens to the changes in GABA content and related substances in oat groats during steeping and germination.

MATERIALS AND METHODS

Oat Materials. Two different naked oat cultivars (*Avena nuda* L.), Bayou I and Baiyan II, were used in the study. The cultivars were all grown in 2008 in bases for growing organic oat, Shanxi, China, and they are the main commercial cultivars in local area. The harvested oat groats were dried to about 10% moisture and the 1000-seed weight 24.63 g and then stored at room temperature until time of steeping and germination, which was performed in the autumn of 2008.

Reagents. γ-Aminobutyric acid (GABA), glutamic acid, amino acid standard solution, and pyridoxal 5-phosphate (PLP) were from Sigma−Aldrich (USA). Sodium citrate buffer solution was Biochrom Ltd. (England). All other chemicals and reagent used in the experiments were of analytical grade.

Steeping and Germination. Dry oat groats were surface-sterilized by dipping in 1% solution of sodium hypochlorite for 30 s and thoroughly rinsed in deionized water before steeping. Seven original samples of oat groats (200 g of each sample) were steeped and germinated in deionized water under controlled conditions in an incubator (HWS-280; Hangzhou Huier Instruments, Zhejiang, China). For each oat cultivar, oat groats were steeped with 1000 mL of deionized water at 20 °C, aeration for 1 h every 4 h, respectively. Three samplings were carried out (S1−S3), which took at 2, 4, and 7 h during the steeping process, respectively. Each of the three samplings corresponded to an estimated moisture level that was determined by comparing fresh and dry weights (1 h at 120 °C) of a subsample. The first sampling (S1) corresponded to approximately 32% moisture, the second sampling (S2) to 37%, and the third sampling (S3) to 44% moisture level. After steeping, the remaining oat groats were drained and germinated for 72 h in a controlled environment at 20 °C and 98% relative humidity, and four samplings were carried out (G1−G4), which took at 7, 13, 25, 37, 48, 60, and 72 h during the germination process. After sampling, samples were immediately freeze-dried (Christ Alpha1-4, Germany) and stored at −40 °C until time of analysis. Just prior to analyses, oat samples were milled with a micro plant grinding machine (FZ102; Tianjin Taisite Instruments, Tianjin, China) set at a fine setting of 0.5 mm. Raw groats were also freeze-dried and used as reference sample in all performed analysis.

Extraction of Free Amino Acids. Free amino acids were extracted according to the method reported previously (*17*) with some modifications. Initially, milled oat sample (2.0 g dry weight (DW)) was defatted two times with 20 mL of hexane at 30 °C by an ultrasonic homogenizer (Scientz-IID, Ningbo Scientz Biotechnology Co., Ltd., Zhejiang, China). The defatted samples were blended with 20 mL of ethanol (75%) and shaken with a laboratory rotary shaker (JB50-D; Shanghai Shengke Instruments, Shanghai, China) at 250 rpm for 30 min at 50 °C, and then the homogenates were centrifuged at 10000*g* for 15 min at 4 °C in a centrifuge (Eppendorf 5417R, Germany). After centrifugation, the ethanol supernatants were removed and extraction was repeated three times at the same conditions. Then supernatants were pooled, vacuum-evaporated to dryness at 40 °C, and reconstituted with 0.2 M sodium citrate loading buffer solution (pH 2.2) to a final volume of 10 mL. The extracts were stored at −40 °C until use.

Determination of γ-Aminobutyric Acid and Glutamic acid. The concentration of free glutamic acid and γ-aminobutyric acid was determined following the method originally described by Rizzello et al. (*18*) with some modifications. Briefly, the free amino acid extracts was filtered through a 0.45 μm of nylon syringe filter (Filtrex Technology, Singapore) prior to analysis and analyzed by a Biochrom 30 series amino acid analyzer (Biochrom Ltd., Cambridge Science Park, England) with a Na-cation-exchange column (8 μm, 4.6 mm ×200 mm). The injection volume was 20 μL, the duration of a single run was 50 min. Amino acids were post-column derivatized with ninhydrin reagent and detected by absorbance at 570 nm. The amino acids extracts and amino acid standard solution including GABA were analyzed under the same conditions, and all of the above experiments were replicated three times. Identification of glutamic acid and γ-aminobutyric acid was performed by comparisons to the retention time and UV spectra of authentic standards from Sigma, then the quantitative data was calculated by their regression equations ($y = 6 \times 10^6 x + 23263$ ($R^2 = 0.9970$) for GABA; $y = 7 \times 10^6 x - 16064$ ($R^2 = 0.9990$) for glutamic acid), as well as other amino acids. Results were expressed as milligrams per 100 g on a dry weight basis (DW).

Determination of GAD Activity. The activity of GAD was assessed according to the method described by Bai et al. (*16*) with some modifications. Two grams of milled, freeze-dried oat groat were mixed, on an ice bath, with 10 mL of potassium phosphate buffer (1/15 M, pH 5.8) containing 2 mM β-mercaptoethanol, 2 mM disodium ethylenediamine tetraacetic acid (EDTA), and 0.2 mM pyridoxal 5-phosphate (PLP) and shaken with a laboratory rotary shaker at 250 rpm for 60 min at 4 °C. Then, the homogenate was centrifuged at 11000*g* for 20 min at 4 °C in a refrigerated high speed centrifuge. The supernatant was the crude GAD. The reaction mixture consisted of 2 mL of crude enzyme liquid and 1 mL of substrate (100 mM glutamic acid). The reaction solution was incubated for 2 h at 40 °C and then terminated for 5 min at 90 °C. The centrifugal suspension was vacuum-evaporated to dryness at 40 °C and reconstituted with 0.2 M sodium citrate loading buffer (pH 2.2) to a final volume of 10 mL. GABA content was analyzed by the GABA determination method, as mentioned above. The activity of glutamate decarboxylase was defined as the nmol amount of GABA produced at 40 °C per min per g on a dry weight basis (*19*).

Statistical Analysis. All experiments were conducted three times independently and the experimental data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's multiple range test were carried out to determine significant differences ($p < 0.05$) between the means by SPSS (version 13.0). Correlation coefficient and regression analyzes were determined by DPS (version 3.01) and EXCEL program.

RESULTS

Changes in GABA Content and GAD Activity. To intuitively compare the changes in the content of GABA and glutamic acid, amino acid analysis chromatogram of extracts from raw groats and sample after 37 h of germination of Baiyan II are shown in **Figure 1**. It can be seen from the figure that GABA and glutamic acid exhibited a significant change between raw groats and germinated oat groats. **Table 1** showed the levels of the GABA and the activities of GAD in cultivars Baiyan II and Bayou I during steeping and germination at 20 °C. There was a significant increase ($p < 0.05$) in the GABA content, as compared to raw groats, and the results for the two cultivars differed to some extent during the steeping and germination process. For Baiyan II, GABA content increased significantly by 7.83-fold at stage S1, thereafter, it first decreased and then increased at the remaining stage of steeping. During germination, A rapid increase ($p < 0.05$) in GABA content was detected, and it increased by 29.41-fold and reached the maximum at stage G6 compared with raw groats (**Table 1**), and then GABA content decreased. For Bayou I, the trend of changes in the GABA content was similar to that in Baiyan II. However, the percentage increase was different at the same test interval, which may come from differences in cultivars. From the studied results, the increase in GABA content seems to have reached a plateau for two cultivars at 60 h of germination, which indicates that the GABA content could not significantly increase even more with further germination.

As compared to raw groats, GAD activity in Baiyan II started to increase from stage S2 and increased by 77.09% ($p < 0.05$) to reach its maximum at the end of steeping. During germination, GAD activity decreased continuously and significantly by 24.47% and reached to the minimum at stage G4, lower than that in raw groats (**Table 1**). Thereafter, GAD activity started to increase, but it had no difference during the later period of germination. In Bayou I, changes of GAD activity during steeping and germination had a

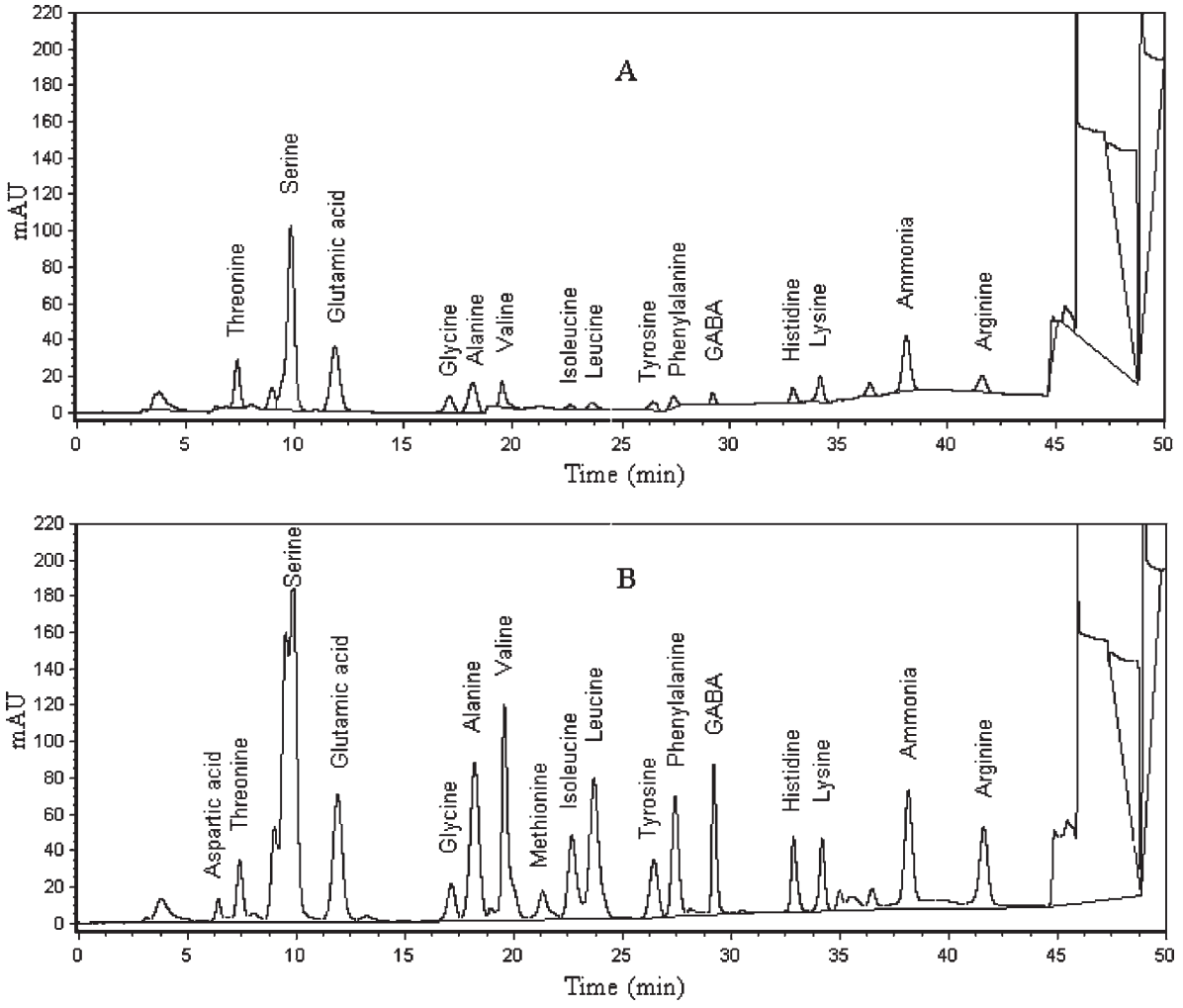

Figure 1. Amino acid analysis chromatograms of the free amino acid extracts from Baiyan II at wavelength 570 nm. Raw groats (**A**) and sample after 37 h of germination (**B**) are shown.

Table 1. Content (mg per 100 g of DW) of GABA, Glutamic Acid, and GAD Activity (nmol GABA per min per g of DW) in Raw Groats and Oat Groats during Steeping and Germination at 20 °C for Baiyan II and Bayou I Cultivars[a]

	Baiyan II			Bayou I		
samples	GABA	GAD	glutamic acid	GABA	GAD	glutamic acid
raw groat	0.54 ± 0.04 h	10.87 ± 0.85 cde	12.29 ± 0.54 f	1.41 ± 0.22 f	13.46 ± 1.22 bc	12.49 ± 0.25 e
S1[b]	4.77 ± 0.20 f	10.71 ± 0.72 cde	8.07 ± 0.35 hi	4.13 ± 0.30 e	13.45 ± 1.14 bc	6.31 ± 0.18 h
S2	3.36 ± 0.24 g	18.46 ± 1.55 a	7.04 ± 0.25 i	3.59 ± 0.24 e	17.46 ± 1.85 ab	5.95 ± 0.15 h
S3	4.73 ± 0.32 f	19.25 ± 1.84 a	8.03 ± 0.38 hi	6.33 ± 0.38 d	9.70 ± 1.05 c	8.81 ± 0.21 g
G1[c]	6.91 ± 0.42 e	15.07 ± 1.20 b	11.84 ± 0.51 fg	6.07 ± 0.26 d	13.11 ± 1.36 c	10.56 ± 0.28 f
G2	5.98 ± 0.45 ef	13.70 ± 1.32 bc	9.96 ± 0.32 gh	5.71 ± 0.35 d	18.73 ± 1.65 a	9.76 ± 0.19 fg
G3	5.95 ± 0.55 ef	10.77 ± 1.10 cde	15.20 ± 0.45 e	7.20 ± 0.41 d	17.80 ± 1.86 a	10.64 ± 0.24 f
G4	8.68 ± 0.48 d	8.21 ± 0.65 e	22.50 ± 0.94 d	10.37 ± 0.62 c	12.96 ± 1.56 c	19.07 ± 0.35 d
G5	11.45 ± 0.52 c	10.50 ± 0.70 de	38.47 ± 1.15 a	15.57 ± 0.75 b	11.12 ± 0.94 c	35.38 ± 0.86 a
G6	16.42 ± 0.63 a	11.74 ± 0.64 cd	30.45 ± 1.12 b	19.62 ± 0.92 a	13.68 ± 1.10 bc	26.26 ± 0.74 b
G7	14.84 ± 0.74 b	12.25 ± 0.44 bcd	27.22 ± 0.84 c	20.35 ± 0.84 a	12.33 ± 1.25 c	24.75 ± 0.58 c

[a] Numbers represent mean values of three independent replicates ± SD. [b] S1−S3 refer to the different steeping stages. [c] G1−G7 refer to the different germination stages. Different letters indicate statistically significant differences between the means ($p < 0.05$) for GABA, glutamic acid, and GAD activity in oat groats of Baiyan II and Bayou I.

similar trend to that of Baiyan II, except for difference in the extent of the change. Interestingly, GAD activity in Baiyan II reached its maximum at stage S3, whereas that in Bayou I was at its minimum at the same stage (S3).

Changes in Glutamic Acid Content. The level of glutamic acid in Baiyan II and Bayou I during steeping and germination is shown in **Table 1**. During steeping, a significant decrease in glutamic acid content was observed and it decreased by 42.72% and 52.36% ($p < 0.05$) and reached their minimum during the stage S2 for Baiyan II and Bayou I, respectively. Glutamic acid content of treated oat groats was not higher than raw groats until stages G3 and G4 for Baiyan II and Bayou I, respectively, in spite of some increases or decreases during this time. Thereafter, glutamic acid first increased by 2.13-fold and 1.83-fold and reached the maximum at stage G5 for Baiyan II and Bayou I, respectively, and then it decreased until the end of germination. Consequently,

Table 2. Content (mg per 1000 g of DW) of Other Amino Acids in Raw Groats and Oat Groats at Some Stages of Steeping and Germination at 20 °C for Baiyan II and Bayou I Cultivars[a]

	Baiyan II				Bayou I			
amino acid	raw groat	S3[b]	G4[c]	G7	raw groat	S3	G4	G7
				Essential				
isoleucine	7.2 ± 0.6	13.5 ± 0.8	128.9 ± 8.8	142.4 ± 9.4	5.8 ± 0.3	16.0 ± 0.8	80.9 ± 2.5	110.5 ± 7.4
leucine	9.7 ± 0.6	26.4 ± 0.6	205.2 ± 9.5	187.5 ± 9.0	9.8 ± 0.6	32.0 ± 1.8	137.2 ± 8.5	152.5 ± 8.0
lysine	23.5 ± 0.8	26.7 ± 0.7	93.8 ± 5.4	95.6 ± 6.0	15.9 ± 0.6	22.0 ± 1.2	51.9 ± 2.1	83.3 ± 3.5
threonine	22.3 ± 1.1	28.7 ± 0.8	85.7 ± 2.4	125.4 ± 8.6	21.0 ± 1.5	31.5 ± 1.8	60.1 ± 2.4	98.3 ± 4.2
valine	23.0 ± 0.9	28.2 ± 0.5	185.2 ± 9.2	177.8 ± 8.8	22.0 ± 1.0	37.3 ± 1.5	125.2 ± 9.6	150.4 ± 9.4
phenylalanine	17.8 ± 0.5	24.6 ± 0.8	167.9 ± 7.6	184.2 ± 9.6	16.1 ± 0.5	32.8 ± 1.7	122.4 ± 6.4	163.5 ± 9.1
histidine	13.4 ± 0.5	15.6 ± 0.8	68.7 ± 6.6	83.4 ± 7.3	16.8 ± 0.6	28.4 ± 1.1	40.7 ± 1.8	61.4 ± 4.3
arginine	27.2 ± 1.0	39.2 ± 1.0	124.0 ± 8.8	145.2 ± 8.7	27.2 ± 0.5	43.5 ± 1.0	91.4 ± 5.8	128.1 ± 9.0
				Nonessential				
glycine	10.9 ± 0.5	18.6 ± 1.1	23.5 ± 1.2	40.6 ± 3.2	11.4 ± 0.5	22.6 ± 1.2	18.1 ± 0.8	36.0 ± 1.4
alanine	29.7 ± 0.7	90.0 ± 2.4	166.7 ± 6.2	154.8 ± 7.6	33.3 ± 0.8	116.1 ± 8.2	146.6 ± 5.6	132.7 ± 8.5
aspartic acid	9.1 ± 0.5	35.3 ± 1.1	54.2 ± 1.8	82.5 ± 3.5	10.9 ± 2.4	38.1 ± 1.8	47.5 ± 1.8	62.6 ± 5.5
tyrosine	16.3 ± 0.5	24.0 ± 0.7	105.3 ± 8.5	136.7 ± 8.4	12.6 ± 0.9	27.8 ± 0.8	76.5 ± 1.6	90.3 ± 7.7
serine	18.6 ± 1.0	29.1 ± 0.5	53.4 ± 1.5	87.0 ± 4.3	15.8 ± 0.6	24.3 ± 0.8	38.9 ± 1.2	49.6 ± 3.2
methionine	nf[d]	9.1 ± 0.4	36.8 ± 1.2	63.1 ± 3.3	nf	7.7 ± 0.4	25.7 ± 1.2	52.0 ± 2.8

[a] Numbers represent mean values of three independent replicates ± SD. [b] S1−S3 refer to the different steeping stages. [c] G4, G7 refer to the different germination stages. [d] nf, not found.

Table 3. Correlation Analysis between GABA, GAD Activity, and Glutamic Acid in Oat Groats during Steeping and Germination for Baiyan II and Bayou I

	Baiyan II				Bayou I			
	glutamic acid		GABA		glutamic acid		GABA	
	R[a]	p[b]	R	p	R	p	R	p
GABA[c]	0.8614	0.0013			0.8874	0.0006		
GAD	−0.5790	0.0795	−0.4520	0.1897	−0.4444	0.1982	−0.3946	0.2592

[a] Correlation coefficient, R. [b] Probability, p. [c] Abbreviations of γ-aminobutyric acid and glutamate decarboxylase (GABA, γ-aminobutyric acid; GAD, glutamate decarboxylase).

glutamic acid content exhibited a similar changing trend in Baiyan II and Bayou I, although they varied with the processing of steeping and germination. In addition, the other amino acids were investigated and their level in raw groats and three stages are shown in **Table 2** (not shown for other stages). These amino acids increased significantly during steeping and germination compared to raw groats.

To further investigate their interrelationship, the correlation between the content of GABA and glutamic acid and GAD activity was established in oat groats during steeping and germination but for raw groats, and correlation coefficients (R) and probability (p) are shown in **Table 3**. During the steeping and germination, there were significantly positive correlations ($p < 0.01$) between the content of GABA and glutamic acid;conversely, GABA and glutamic acid were low correlated ($p > 0.05$) to the GAD activity for two cultivars.

DISCUSSION

In the current study, GABA in oat was accumulated by steeping and germination, similar to the observations on brown rice (*6*, *14*). GAD reaction may be stimulated with an increase of glutamic acid (*11*, *20*). Namely, glutamic acid content would decrease with the accumulation of GABA, which was only observed during the first stage of steeping in present study (**Table 1**). However, correlations between GABA and glutamic acid for two cultivars in the current study were in disagreement with the report mentioned above (**Table 3**). GABA is mainly from the conversion of glutamic acid, while glutamic acid is mainly derived from the breakdown of protein on germination (*21*). In addition, glutamic acid may be increased by the glutamine synthetase−glutamate synthase cycle (*10*, *22*) and provided by GABA transaminase reactions (*10*). At the same time, glutamic acid and GABA are consumed for protein synthesis as a means of recycling arginine-derived nitrogen and carbon (*22*). Therefore, one explanation for this result in the present study is probably that glutamic acid is being produced while being converted and that the conversion of glutamic acid to GABA may have some influence on its production in oat groats during steeping and germination, namely the higher the conversion, the more production relatively at the time. Makno et al. (*23*) reported that the relationship between GABA and glutamic acid is unclear in the study on vine-ripe tomato (*Lycopersicon esculentum* Mill.) fruits under modified atmospheres.

In general, the accumulation of GABA is higher when GAD activity increased and vice versa. The activity of GAD of yeast showed a linear increase with increasing substrate−glutamic acid concentration (*24*). The result reaserched by Liu et al (*25*) showed that there was a significantly positive correlation between the GABA content and GAD activity. Sadami et al. (*26*) reported that the GAD activity of rice germ was dependent on the substrate concentration and reached a maximal at 50−100 mM glutamic acid. Komatsuzaki et al. (*27*) suggests that GAD activity and GABA content were regulated by glutamic acid addition. It seems plausible that the GAD activity was raised as the germination continued, and thus GABA was continually produced. However, the relationship between GABA and GAD activity was not found ($p > 0.05$), as well as between glutamic acid and GAD activity (**Table 3**). Consequently, these differences mentioned above may be related to the complex metabolic pathways involved, materials from different origins and various environmental stress, which will also need to be further investigated. So these results in the present study apply only for the two oats cultivars studied.

In the present study, although the GABA contents in steeped or germinated oats were much higher than that in raw groats and

waxy hull-less barley (*11*) (2.2−8.6 mg/100 g of DW), they were lower than some reports in brown rice (*6*) (11.02−149.03 mg/100 g of DW) and foxtail millet (*16*) (42.9 mg/100 g of fresh weight under the optimal conditions). However, it was difficult to compare the GABA content between the present and previous studies due to different species and processing of steeping and germination, which indicates that more work need be performed on the steeping and germination process to optimize the increase of the GABA. In addition, Inoue et al. (*28*) reported that 10−12 mg of GABA for 12 weeks to people with mild hypertension was effective for lowering their systolic and diastolic blood pressures by 17.4 ± 4.3 and 7.2 ± 5.7 mmHg. The dose is equivalent to 50−80 g oat groats after 60 h of germination, which suggests that steeping and germination is useful for increasing the antihypertensive effect of oat groats.

In conclusion, the current study indicates that steeping and germination of oats under highly controlled conditions can accumulate GABA in oat groats. The choice of stages of steeping and germination might be of great importance. However, the relationship between the activity of GAD, glutamic acid, and GABA content is unclear because of the complex metabolic pathways, more assays need to be further performed with more cultivars (oats and others).

ABBREVIATIONS USED

GABA, γ-aminobutyric acid; GAD, glutamate decarboxylase; PLP, pyridoxal 5-phosphate; EDTA, disodium ethylenediamine tetraacetic acid; DW, dry weight of sample.

ACKNOWLEDGMENT

We thank Doctor Meng Zhao for his technical assistance in amino acids analysis.

LITERATURE CITED

(1) Satyanarayan, V.; Nair, P. M. Metabolism, enzymology and possible role of 4-aminobutyrate shunt in higher plants. *Phytochemistry* **1990**, *29*, 367–375.

(2) Roberts, E. γ-Aminobutyric acid and nervous system functions—a perspective. *Biochem. Pharmacol.* **1974**, *23*, 2637–2649.

(3) Yoshimura, M.; Toyoshi, T.; Sano, A.; Izumi, T.; Fujii, T.; Konishi, C.; Inai, S.; Matsukura, C.; Fukuda, N.; Ezura, H.; Obata, A. Antihypertensive effect of a γ-aminobutyric acid rich tomato cultivar "DG03-9" in spontaneously hypertensive rats. *J. Agric. Food Chem.* **2010**, *58*, 615–619.

(4) Oh, S. H.; Soh, J. R.; Cha, Y. S. Germinated brown rice extract shows a nutraceutical effect in the recovery of chronic alcohol-related symptoms. *J. Med. Food.* **2003**, *6*, 115–121.

(5) Oh, C.-H.; Oh, S.-H. Effect of germinated brown rice extracts with enhanced levels of GABA on cancer cell proliferation and apoptosis. *J. Med. Food* **2004**, *7*, 19–23.

(6) Komatsuzaki, N.; Tsukahara, K.; Toyoshima, H.; Suzuki, T.; Shimizu, N.; Kimura, T. Effect of soaking and gaseous treatment on GABA content in germinated brown rice. *J. Food Eng.* **2007**, *78*, 556–560.

(7) Jeng, K.-C.; Chen, C.-S.; Fang, Y.-P.; Hou, R. C.-W.; Chen, Y.-S. Effect of microbial fermentation on content of statin, GABA, and polyphenols in Pu-Erh tea. *J. Agric. Food Chem.* **2007**, *55*, 8787–8792.

(8) Nogata, Y.; Nagamine, T. Production of free amino acids and γ-aminobutyric acid by autolysis reactions from wheat bran. *J. Agric. Food Chem.* **2009**, *57*, 1331–1336.

(9) Xing, S. G.; Jun, Y. B.; Hau, Z. W.; Liang, L. Y. Higher accumulation of γ-aminobutyric acid induced by salt stress through stimulating the activity of diamine oxidases in *Glycine max* (L.) Merr. roots. *Plant Physiol. Biochem.* **2007**, *45*, 560–566.

(10) Bouché, N.; Fromm, H. GABA in plants: just a metabolite. *Trends in Plant Sci.* **2004**, *9*, 111–115.

(11) Chung, H.-J.; Jang, S.-H.; Cho, H. Y.; Lim, S.-T. Effects of steeping and anaerobic treatment on GABA (γ-aminobutyric acid) content in germinated waxy hull-less barley. *LWT—Food Sci. Technol.* **2009**, *42*, 1712–1716.

(12) Kinnersley, A. M.; Turano, F. J. γ-Aminobutyric acid (GABA) and plant responses to stress. *Crit. Rev. Plant Sci.* **2000**, *19*, 479–509.

(13) Kaukovirta-Norja, A.; Wilhelmsson, A.; Poutanen, K. Germination: a means to improve the functionality of oats. *Agric. Food* **2004**, *13*, 100–112.

(14) Shu, X.-L.; Thomas, F.; Shu, Q.-Y.; Engel, K.-H. Metabolite profiling of germinating rice seeds. *J. Agric. Food Chem.* **2008**, *56*, 11612–11620.

(15) Xu, J. G.; Tian, C. R.; Hu, Q. P.; Luo, J. Y.; Wang, X. D.; Tian, X. D. Dynamic changes in phenolic compounds and antioxidant activity in oats (*Avena nuda* L.) during steeping and germination. *J. Agric. Food Chem.* **2009**, *57*, 10392–10398.

(16) Bai, Q.; Chai, M.; Gu, Z.; Cao, X.; Li, Y.; Liu, K. Effects of components in culture medium on glutamate decarboxylase activity and γ-aminobutyric acid accumulation in foxtail millet (*Setaria italica* L.) during germination. *Food Chem.* **2009**, *116*, 152–157.

(17) Lin, L.-Y.; Peng, C.-C.; Yang, Y.-L.; Peng, R. Y. Optimization of bioactive compounds in buckwheat sprouts and their effect on blood cholesterol in hamsters. *J. Agric. Food Chem.* **2008**, *56*, 1216–1223.

(18) Rizzello, C. G.; Cassone, A.; Di Cagno, R.; Gobbetti, M. Synthesis of angiotensin I-converting enzyme (ACE)-inhibitory peptides and γ-aminobutyric acid (GABA) during sourdough fermentation by selected lactic acid bacteria. *J. Agric. Food Chem.* **2008**, *56*, 6936–6943.

(19) Zhang, H.; Yao, H.-Y.; Chen, F.; Wang, X. Purification and characterization of glutamate decarboxylase from rice germ. *Food Chem.* **2007**, *101*, 1670–1676.

(20) Saikusa, T.; Horino, T.; Mori, Y. Distribution of free amino acids in the rice kernel and kernel fractions and the effect of water soaking on the distribution. *J. Agric. Food Chem.* **1994**, *42*, 1122–1125.

(21) Streeter, J. G.; Thompson, J. F. Anaerobic accumulation of γ-aminobutyric acid and alanine in radish leaves (*Raphanus sativus* L.). *Plant Physiol.* **1972**, *49*, 572–578.

(22) Shelp, B. J.; Bown, A. W.; McLean, M. D. Metabolism and functions of gamma-aminobutyric acid. *Trends Plant Sci.* **1999**, *4*, 446–452.

(23) Makno, Y.; Soga, N.; Oshita, S.; Kawagoe, Y.; Tanaka, A. Stimulation of γ-aminobutyric acid production in Vine-Ripe tomato (*Lycopersicon esculentum* Mill.) fruits under modified atmospheres. *J. Agric. Food Chem.* **2008**, *56*, 7189–7193.

(24) Tong, J. C.; Mackay, I. R.; Chin, J.; Law, R. H. P.; Fayad, K.; Rowley, M. J. Enzymatic characterization of a recombinant isoform hybrid of glutamic acid decarboxylase (rGAD67/65) expressed in yeast. *J. Biotechnol.* **2002**, *97*, 183–190.

(25) Liu, L. L.; Zhai, H. Q.; Wan, J.-M. Accumulation of γ-aminobutyric acid in giant-embryo rice groat in relation to glutamate decarboxylase activity and its gene expression during water soaking. *Cereal Chem.* **2005**, *82*, 191–196.

(26) Sadami, O.; Satoshi, A.; Kazuhito, S.; Isao, M. Enzymatic production of caminobutyric acid using rice (*Oryza sativa*) germ. *Food Sci. Technol. Res.* **2000**, *6*, 208–211.

(27) Komatsuzaki, N.; Shima, J.; Kawamoto, S.; Momose, H.; Kimura, T. Production of γ-aminobutyric acid (GABA) by *Lactobacillus paracasei* isolated from traditional fermented foods. *Food Microbiol.* **2005**, *22*, 497–504.

(28) Inoue, K.; Shirai, T.; Ochiai, H.; Kasao, M.; Hayakawa, K.; Kimura, M.; Sansawa, H. Blood-pressure-lowering effect of a novel fermented milk containing γ-aminobutyric acid (GABA) in mild hypertensives. *Eur. J. Clin. Nutr.* **2003**, *57*, 490–495.

Received for review April 4, 2010. Revised manuscript received July 28, 2010. Accepted July 28, 2010. This work was supported by a project of the Natural Science Foundation for Young Scientists of Shanxi Province, China (project no. 2008021040), the Shanxi Soft Science Research Program (project no. 2010041031-02), and a project of Scientific and Technological Development for universities and colleges in Shanxi (project no. 2010112).