



Effect of the treatment by slightly acidic electrolyzed water on the accumulation of γ -aminobutyric acid in germinated brown millet



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ABSTRACT

The accumulation of γ -aminobutyric acid and the microbial decontamination are concerned increasingly in the production of sprouts. In this work, the effect of the treatment by slightly acidic electrolyzed water on the accumulation of γ -aminobutyric acid in the germinated brown millet was evaluated by high performance liquid chromatography during germination. The results showed that slightly acidic electrolyzed water with appropriate available chlorine (15 or 30 mg/L) could promote the accumulation of γ -aminobutyric acid by up to 21% ($P < 0.05$). However, the treatment with slightly acidic electrolyzed water could not enhance the sprouts growth of the germinated brown millet. The catalase and peroxidase activities of the germinated brown millet during germination were in agreement with the sprouts growth. Our results suggested that the accumulation of γ -aminobutyric acid was independent of the length of sprouts in germinated grains. Moreover, the treatment with slightly acidic electrolyzed water significantly reduced the microbial counts in the germinated millet ($P < 0.05$) and the treatment with high available chlorine concentration (15 and 30 mg/L) showed stronger anti-infection potential in the germinated brown millet than that of lower available chlorine concentration (5 mg/L). In conclusion, the treatment with slightly acidic electrolyzed water is an available approach to improve the accumulation of γ -aminobutyric acid and anti-infection potential in the germinated brown millet, and it can avoid too long millet sprouts.

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1. Introduction

Admittedly, γ -aminobutyric acid, a non-protein amino acid, has showed many healthy benefits on humans and animals such as an inhibitor of neurotransmitter, hypotensive and anticancer (Diana, Quilez, & Rafecas, 2014; Ishikawa, Watabe, & Goto, 1983; Nicholson-G, Guthrie, Sutton, & Baenziger, 2001; Suwanmanon & Hsieh, 2014). As an endogenous phytochemical, γ -aminobutyric acid could be found in many plants with a very low content ranged from 0.3 to 32.5 $\mu\text{mol/g}$ (Song et al., 2005; Xu & Hu, 2014). Therefore, the accumulation of γ -aminobutyric acid is concerned increasingly in some plants especially used as food materials.

Foxtail millet (*Setaria italica*), originated from China, is one of the most important food crops in the semi-arid tropics, and now

planted all over the world (Amadou, Le, Amza, Sun, & Shi, 2013). As a good source of cereals, the millet has been recognized as one of the highly nutritious among cereals and contains a large amount of bioactive ingredients (Devisetti, Yadahally, & Bhattacharya, 2014; Shahidi & Chandrasekara, 2013; Veenashri & Muralikrishna, 2011; Zhang & Liu, 2015). The sprouting millet was also a potential source of functional food and used to produce the food and beverages for young children and adults, such as beer, weaning and geriatric food formulations (Amadou et al., 2013; Shahidi & Chandrasekara, 2013).

During the germination, many significant biochemical and physical reactions could undesirably reduce the substances and promote the edible values of sprouts (Li, Chen, Yao, & Xu, 2007). For instance, the germinated brown rice contained much more γ -aminobutyric acid than the raw brown rice (Chung, Cho, & Lim, 2012). Moreover, soaking and germination are effective methods to promote the accumulation of γ -aminobutyric acid in some plant

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seeds such as brown rice, beans, oats, millet, barely and so on (Bai et al., 2009; Chung, Jang, Cho, & Lim, 2009; Liu et al., 2013; Xu & Hu, 2014). However, the accumulation of γ -aminobutyric acid in the germinated brown millet was not reported in the previous studies. In addition, how to avoid the microbial contamination during the germination is increasingly concerned by consumers and scientists.

In the past years, due to its neutral pH (5.0–6.5) and lower available chlorine concentration (5–30 mg/L), the slightly acidic electrolyzed water (SAEW) with high anti-infection potential for agricultural products had shown a promising prospect in food industries (Ding et al., 2015; Issa-Zacharia, Kamitani, Miwa, Muhimbula, & Iwasaki, 2011). Our previous study found that SAEW was effective on reducing the microbial counts on the brown rice and enhanced the accumulation of γ -aminobutyric acid in the germinated brown rice (Liu et al., 2013). Moreover, SAEW was demonstrated to not only reduce the microbial counts on the surface of mung bean sprouts, but also promote the growth of sprouts in our previous study (Rui, Jianxiong, Haijie, & Lite, 2011).

In the present study, the effect of the treatment by SAEW on the accumulation of γ -aminobutyric acid in GBM was investigated during germination. Considering that the sprouts length maybe influence on the GABA contents, the impact of SAEW on the growth of germinated millet was also investigated. Moreover, the efficacy of SAEW on reducing microbial counts on GBM during germination was evaluated. The objective of this study is to evaluate the effect of SAEW on the accumulation of GABA and anti-infection potential in GBM production.

2. Materials and methods

2.1. Materials

The cultivar “Jigu 19” of foxtail millet (*S. italica*) used in this study was generously provided by the Institute Millet Crops of Hebei Academy of Agriculture and Forestry of China. The millet was harvested on September 30th of 2013. The dried millet was dehulled in an experimental rubber roll sheller (THU class 35A, Satake rice machine, Tokyo, Japan). The millet was sealed in plastic bags and stored at 4 °C until used.

Standard γ -aminobutyric acid was purchased from Sigma (MO, USA). Analytical grade chemicals and distilled water were used in the present study.

2.2. Preparation of SAEW

The original SAEW was prepared using a flow type electrolysis apparatus (Model AQUACIDO NDX-250KMS, OSG Company Ltd., Japan). The diluted SAEW was prepared with distilled water. In this study, SAEW was stored in polypropylene containers and immediately used for the measurement. The pH and ORP of SAEW were measured by a pH/ORP meter (Model 86802, Orion Inc. America)

and the ACC of SAEW was measured by the iodometric method. The pH, ORP and ACC of all the treatment solutions are shown in Table 1.

2.3. Production of germinated brown millet

Three hundred grams of dehulled millet were washed for 2 h with 3000 mL treatment solutions (different SAEW shown in Table 1 and tap water (TW) as control). After washing, the millet was soaked in the treatment solution (1:3, m/v) for 12 h. The soaked samples were then drained and spread on sterile cheese-cloth in a plastic box with holes in the bottom in a HWS constant temperature humidity Chamber (Ningbo, China) at 25 °C with a humidity of 85–90%. The same treatment solutions (1000 mL) were used to water the treated germinated millet once per day for 3 days.

The γ -aminobutyric acid contents, morphological measurements, catalase (CAT) and peroxidase (POD) activities and microbiological analysis were evaluated at 0, 12, 24, 48 and 72 h, respectively.

2.4. Determination of γ -aminobutyric acid content in the germinated brown millet

The germinated brown millet was freeze-dried and ground to powder. The freeze-dried powder sample (0.500 g) was mixed with 5 mL 10% trichloroacetic acid, and γ -aminobutyric acid was extracted by shaking on an oscillator for 1 min and then held at 40 °C for 2 h. The extracts were centrifuged at 10,000 rpm for 15 min and the supernatants were filtered through a 0.45 mm filter. The content of γ -aminobutyric acid in the supernatants was determined by HPLC method (Meng et al., 2008).

2.5. Morphological measurements of GBM

The length of the sprouts on the germinated millet was measured by a Vernier caliper, and 30 sprouts from each treatment were used.

2.6. Extraction and assay of catalase activity of GBM

The extraction and assay of CAT activity of GBM were performed as described by EA and McHale NA with some slight modification (Havir & McHale, 1987). In brief, 2 g of germinated millet were mixed with 5 mL chilling phosphate buffer (pH 7.5, 0.1 mol L⁻¹) which contained 5 mmol L⁻¹ dithiothreitol and 20 g/L PVP and then ground at the ice bath. The mixtures were centrifuged at 10,000 rpm for 20 min at 4 °C and the supernatant was used as the crude enzymes for the determination of CAT activity. 3 mL of 10 mmol/L H₂O₂ and 200 μ L crude enzymes were mixed and the absorbance at 240 nm was determined. One unit of CAT activity was defined as the amount of enzyme that caused an

Table 1
Physical and chemical parameters of different solutions used in the present study.^{a,b}

Treatment solutions	pH	ORP (mV)	ACC (mg/L)
TW (tap water) ^c	7.69 \pm 0.06	370 \pm 6	ND ^d
Slightly acidic electrolyzed water 1 (SAEW1)	5.85 \pm 0.05	815 \pm 12	5.37 \pm 0.35
Slightly acidic electrolyzed water 2 (SAEW2)	5.90 \pm 0.02	845 \pm 13	15.46 \pm 0.32
Slightly acidic electrolyzed water 3 (SAEW3)	5.96 \pm 0.07	834 \pm 8	30.35 \pm 0.46

^a Values represented the mean \pm SD (n = 5); the observed temperature and atmospheric pressure were 23 \pm 2 °C and 760 \pm 3 mmHg, respectively.

^b Tap water was the drinking water that came from Hebei University of Science and Technology.

^c ND, no chlorine was detected via the iodometric titration method.

^d ORP was the abbreviation of oxidizing reducing potential; ACC was the abbreviation of available chlorine concentration.

increase in absorbance of 0.01 at 240 nm in 1 min. Enzyme activity was expressed as U/g min^{-1} .

2.7. Extraction and assay of peroxides activity of GBM

The extraction and assay of POD activity were performed as described by Soto and Gardea with some slight modification (Soto-Zamora, Yahia, Brecht, & Gardea, 2005). Briefly, 2 g of the germinated millet were mixed with 5 mL of acetate buffers (pH 5.5, 0.1 mol L^{-1}) which contained 20 g/L of PVPP, 1 mmol L^{-1} of polyethylene glycol 6000, 1 mmol L^{-1} of phenylmethylsulfonyl fluoride (PMSF) and 0.01% (v/v) of TritonX-100. The mixtures were ground at the ice bath and then centrifuged at 10,000 rpm for 20 min at 4°C . The supernatants were used as crude enzymes extract for the determination of POD activity. 4.2 mL of acetic acid buffers (0.05 mol L^{-1} , pH 5.5), 0.2 mL of guaiacol (0.04 mol/L), 0.2 mL H_2O_2 (0.15 mol/L) and 0.4 mL of crude enzymes were mixed as the reaction system for the determination of POD activity. The absorbance at 460 nm was measured and one unit of POD activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01 at 460 nm in 1 min. POD activity was expressed as U/g min^{-1} .

2.8. Microbiological analysis

To enumerate the microorganisms, 10 g of millet sample was homogenized for 3 min on a homogenizer. The homogenate was combined with 90 mL of sterile 0.85% sodium chloride solution and agitated for 2 min at low speed. The aliquot was used for various serial dilutions. The total bacteria counts were determined by spreading 0.1 mL of diluted sample onto Plate Count Agar (Aoboxing Bioscience Inc., Beijing, China). The plates were incubated at 35°C for 48 h and the colonies were counted. In this study, the microorganism counts of germinated millet samples were expressed in $\log_{10}\text{CFU g}^{-1}$.

2.9. Statistical analysis

Each treatment was repeated 3 times. For each treatment, data from independent replicate trials were pooled. The means and the standard deviations were calculated. All data were analyzed using Duncan's multiple range test (SPSS16.0 for Windows, SPSS Inc.,

Chicago USA). Significant differences among the treatments were established at a significant level of $P < 0.05$.

3. Results

3.1. Effect of SAEW on GABA accumulation of GBM during germination

The effect of SAEW on the accumulation of GABA in GBM during germination was evaluated and the results are shown in Figs. 1 and 2. Fig. 1 presented the HPLC chromatogram for determining the content of GABA. As shown in Fig. 2, the contents of γ -aminobutyric acid in the germinated millet showed a slightly increasing tendency during the germination. The contents of γ -aminobutyric acid in SAEW1-germinated millet are always lower than that of SAEW2, SAEW3 and TW, which implied that SAEW with low ACC (about 5 mg/L) treatment has adverse impacts on the accumulation of γ -aminobutyric acid. During the early germination (0–48 h), SAEW2 and SAEW3 treatments did not show the significant advantage compared with TW treatment as control. However, the contents of γ -aminobutyric acid in the germinated millets treated by SAEW2 and SAEW3 reached up to 31.3 and 30.9 mg/100 g at the late period (72 h), respectively, which are significantly higher than that of TW treatment of 25.4 mg/100 g ($P < 0.05$). The results showed that SAEW treatment with appropriate ACC of 15 or 30 mg/L could significantly promote the accumulation of GABA by up to 21% ($P < 0.05$).

3.2. Effect of SAEW on the length of millet sprout during germination

The effects of SAEW on the length of the millet sprout during the germination were evaluated and the results are shown in Table 2 and Fig. 3. Fig. 3 showed the appearance of GBM. At the early germination (0–12 h), the millet sprouts of all the treatments were not detectable by naked eyes. After germinated for 24 h, the millet sprouts with TW treatment were observed (about 1.02 mm), while that of three SAEW treatments still were not detected. At the late germination (48–72 h), the millet sprouts of all the treatments were observed obviously. The lengths of millet sprouts treated by SAEW1, SAEW2 and SAEW3 after 48 h germination reached up to 0.71, 0.81 and 0.88 mm, respectively; while the length of the control was 1.20 mm. There were no significant differences existed at

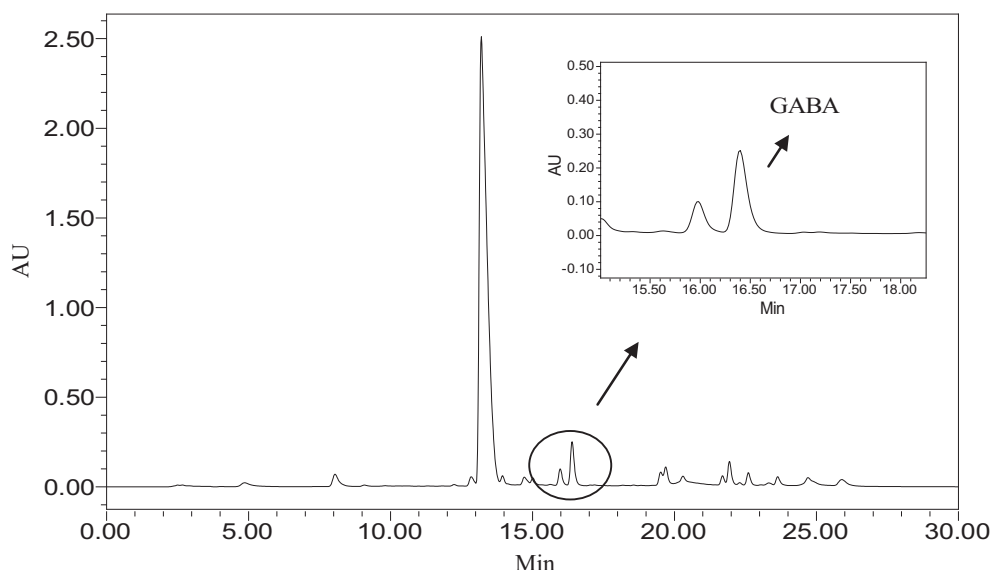


Fig. 1. The HPLC chromatogram for determining the content of γ -aminobutyric acid (GABA) in germinated brown millet (GBM).

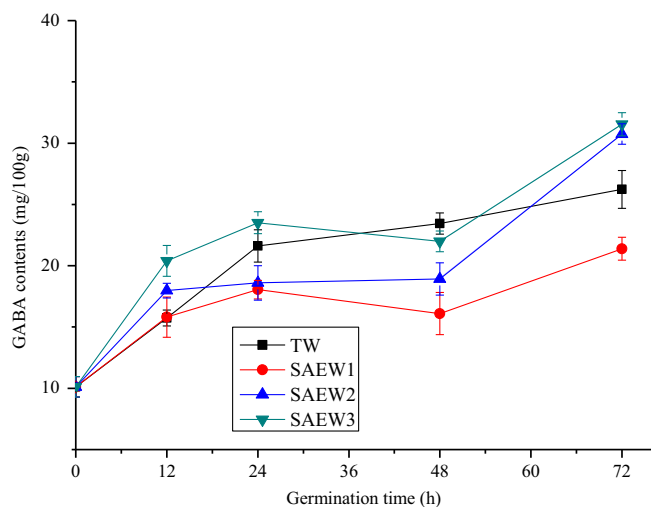


Fig. 2. The changes of γ -aminobutyric acid (GABA) contents of millet treated by slightly acidic electrolyzed water (SAEW) during germination. Four treatments were conducted as follows: (1) tap water (TW) treatment as control; (2) SAEW1 treatment; (3) SAEW2 treatment; (4) SAEW3 treatment. Each treatment was repeated three times and data were expressed by mean \pm standard deviation. The physical and chemical parameters of SAEW and TW used in the experiment were shown in Table 1.

Table 2
The length of millet sprouts during germination of millet treated by SAEW.^{a,b,c,d}

Germination time (h)	Length of millet sprouts (mm)			
	TW	SAEW1	SAEW2	SAEW3
0	0a	0a	0a	0a
12	NDa	NDa	NDa	NDa
24	1.02 \pm 0.12b	NDa	NDa	NDa
48	1.20 \pm 0.11a	0.70 \pm 0.12c	0.81 \pm 0.14bc	0.88 \pm 0.10b
72	3.48 \pm 0.23a	1.86 \pm 0.09c	1.36 \pm 0.27b	2.20 \pm 0.33d

^a SAEW was the abbreviation of slightly acidic electrolyzed water and TW was the abbreviation of tap water; the physical and chemical parameters of SAEW and TW used in the experiment were shown in Table 1.

^b Data were expressed by mean \pm standard deviation (SD) and values were obtained by three replicated measurements.

^c Different letters indicate significant differences ($P < 0.05$) in row and comparison of means were formed using Duncan's multiple comparison tests.

^d ND, no millet sprouts was observed by naked eyes and the length of sprouts was not detected by vernier caliper.

SAEW2 and SAEW3, SAEW2 and SAEW1 ($P < 0.05$). However, the lengths of millet sprouts treated by all the SAEW were significantly lower than that of TW ($P < 0.05$). The similar results were obtained at 72 h during germination and the millet sprouts of TW, SAEW1, SAEW2 and SAEW3 treatments were 3.48, 1.86, 1.36 and 2.20 mm, respectively. Similarly, the lengths of millet sprouts treated by all the SAEW were significantly lower than that of TW ($P < 0.05$).

3.3. Effect of SAEW on the catalase and peroxidase activities of GBM

To demonstrate the sprouts growth of the germinated millet treated by SAEW, the catalase and peroxidase activities during the germination were evaluated and the results are shown in Figs. 4 and 5. From Fig. 4, the catalase activities of the germinated millet treated by all three SAEW were obviously lower than that of TW treatment after 12 h germination. After further germination (24–72 h), there were no significant differences among the catalase activities of germinated millet treated by all the treatments ($P < 0.05$). Moreover, the peroxidase activities of the germinated

millet treated by all the treatments first increased and then decreased during the germination (Fig. 5). However, After 12 h germination, the peroxidase activity of the germinated millets treated by SAEW2 and SAEW3 reached up to the highest point, which were significantly higher than that of the control group. After 24 h germination, the POD peaks of SAEW1-germinated millet just occurred and were higher than that of TW.

3.4. Effect of SAEW on the total bacterial counts of millet during germination

As shown in Table 3, the efficacies of SAEW treatment dipping on reducing the microbial populations on the germinated millets were investigated. The initial microbial populations of total aerobic bacteria were 7.37 ($\log_{10}\text{CFU g}^{-1}$). The microbial populations of millet treated by SAEW1, SAEW2 and SAEW3 for 2 h decreased to 6.57, 6.30 and 6.61 $\log_{10}\text{CFU g}^{-1}$, respectively in comparison with that of the control (7.72 $\log_{10}\text{CFU g}^{-1}$). The results showed that TW treatment resulted in increasing microbial counts compared with the initial load. There are many microbial populations (about 8.15 $\log_{10}\text{CFU g}^{-1}$) existed in the local TW (not shown in data), which could lead to the microbial cross contamination when TW was used in food processing (Liu et al., 2013). Our results found that SAEW treatment could reduce the microbial populations in millets dipped for 2 h by 0.76–1.07 $\log_{10}\text{CFU g}^{-1}$. The disinfection efficacy of SAEW on food stuff had been demonstrated by the previous studies (Issa-Zacharia et al., 2011; Rui et al., 2011).

When producing sprouts, the microorganism contamination during the germination is concerned increasingly by the consumers. The total bacterial counts in the millets treated by SAEW during the germination were also investigated (Table 3). Generally, in all groups, the microbial populations in the millets increased during the germination compared with that of millet dipped for 2 h. However, the results showed that all the SAEW treatments could reduce the total microbial counts in compared with the control at 24, 48 and 72 h, respectively.

After 24 h germination, the total microbial populations in millets treated by SAEW1, SAEW2 and SAEW3 reached to 7.41, 7.27 and 7.26 $\log_{10}\text{CFU g}^{-1}$, respectively; while the total microbial population with TW treatment was 8.38 $\log_{10}\text{CFU g}^{-1}$. SAEW2 and SAEW3 treatments have better disinfection efficacies than that of SAEW1 group ($P < 0.05$). However, there were no significant differences between SAEW2 and SAEW3 treatments. The main difference among these three SAEW was the ACC (Table 1). The results showed that SAEW with higher ACC (15 and 30 mg/L) has stronger disinfection efficacy than that of lower ACC (5 mg/L), which is in agreement with the previous work (Hao et al., 2012). The similar results were obtained after 48 and 72 h germination (Table 3). Generally, current results suggested the all the SAEW treatments could effectively reduce the risk of the microorganism contamination during the germination of millet compared with TW treatment as control, which is consistent with the previous work (Liu et al., 2013).

4. Discussion

There are higher content of γ -aminobutyric acid in the germinated grains than that in the raw grains (Chung et al., 2009). Our results showed that SAEW treatment with appropriate ACC (about 15 or 30 mg/L) could promote the accumulation of γ -aminobutyric acid in the millet during germination, which is in agreement with the previous reports (Chung et al., 2009). High levels of γ -aminobutyric acid can be accumulated in plant tissues under various adverse conditions, such as water stress, and salt stress (Veeranagamallaiah et al., 2008). As for the germination,



(A) Germinated brown millet (Germination for 48 h)



(B) Germinated brown millet (Germination for 72 h)

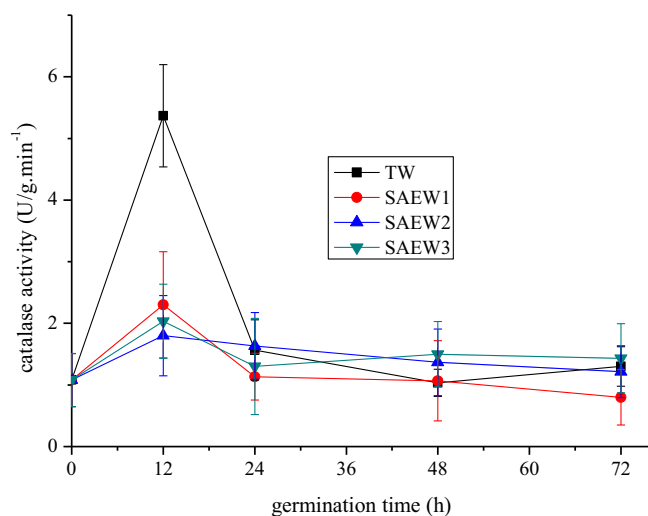
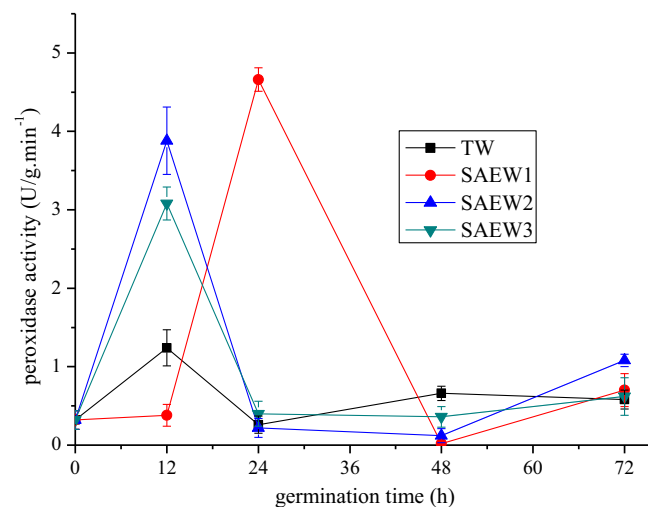
Fig. 3. The photo of the appearance of germinated brown millet (GBM).**Fig. 4.** The changes of catalase activity of millet treated by slightly acidic electrolyzed water (SAEW) during germination. Four treatments were conducted as follows: (1) tap water (TW) treatment as control; (2) SAEW1 treatment; (3) SAEW2 treatment; (4) SAEW3 treatment. Each treatment was repeated three times and data were expressed by mean \pm standard deviation. The physical and chemical parameters of SAEW and TW used in the experiment were shown in Table 1.**Fig. 5.** The changes of peroxidase activity of millet treated by slightly acidic electrolyzed water (SAEW) during germination. Four treatments were conducted as follows: (1) tap water (TW) treatment as control; (2) SAEW1 treatment; (3) SAEW2 treatment; (4) SAEW3 treatment. Each treatment was repeated three times and data were expressed by mean \pm standard deviation. The physical and chemical parameters of SAEW and TW used in the experiment were shown in Table 1.

Table 3The total bacterial counts of millet dipped into SAEW and TW during germination.^{a,b,c}

Treatments	Total bacterial counts of millet during germination (log ₁₀ cfu/g)				
	0 h	2 h	24 h	48 h	72 h
TW	7.37 ± 0.21	7.72 ± 0.05a	8.38 ± 0.21a	8.42 ± 0.03a	8.38 ± 0.22a
SAEW1		6.57 ± 0.04b	7.41 ± 0.02b	7.70 ± 0.23b	7.62 ± 0.12b
SAEW2		6.30 ± 0.11c	7.27 ± 0.12c	7.52 ± 0.09b	7.47 ± 0.13c
SAEW3		6.61 ± 0.10b	7.26 ± 0.07c	7.36 ± 0.13c	7.41 ± 0.15c

^a SAEW was the abbreviation of slightly acidic electrolyzed water and TW was the abbreviation of tap water; the physical and chemical parameters of SAEW and TW used in the experiment were shown in Table 1.

^b Data were expressed by mean ± standard deviation (SD) and values were obtained by three replicated measurements.

^c Different letters indicate significant differences ($P < 0.05$) in line and comparison of means were formed using Duncan's multiple comparison tests.

SAEW also could be supposed to a kind of stress, which may explain the reason of the accumulation of γ -aminobutyric acid in SAEW-germinated millet. In plants, stress initiates a signal transduction pathway where the accumulation of γ -aminobutyric acid probably is mediated primarily by glutamate decarboxylase, as well as γ -aminobutyric acid transaminase, succinic semialdehyde dehydrogenase and the intracellular and intercellular transportation (Song et al., 2005). Moreover, it was reported that the accumulation of γ -aminobutyric acid in germinated grains is related to the activation of GAD during the germination (Bai et al., 2014). However, the changes of GAD during the germination of millets by SAEW are still unknown in the present study. The mechanism on the relationship of GAD and γ -aminobutyric acid in SAEW-germinated millet will be further studied in our research.

As shown in Table 2, our results found SAEW treatments could not promote the sprouts growth of germinated millet in comparison with the control group. However, SAEW could improve the sprouts growth of mung beans and brown rice (Liu et al., 2013; Rui et al., 2011). It was suggested that this phenomenon is mainly caused by the species difference among the millet (used in the present study), mung beans and brown rice (used in the previous study). The mung bean sprouts are used directly as foodstuff while the millet sprouts only as similar to the brown rice. In addition, the particle size of millet is much smaller than that of rice, thus the too long millet sprouts are not wanted in the GBM processing.

CAT and POD played important roles in the germination of seeds. The adverse effect of activated oxygen species such as superoxide radicals and hydrogen peroxide on cellular components has been studied in recent years. All the organisms exposed to an aerobic environment must have some protective mechanism against such harmful oxygen species. Enzymes, such as CAT and POD, are known to play an important role in the scavenging system of activated oxygen (Tanida, 1996). Low CAT activity and high POD activity would lead to the accumulation H₂O₂ as well as promote the cell to disassemble (Martínez-Castellanos, Shirai, Pelayo-Zaldívar, Pérez-Flores, & Sepúlveda-Sánchez, 2009). The results obtained from the current study demonstrated our above study on the sprouts growth.

The strong anti-infection efficacy of SAEW has been demonstrated in many products such as fresh-cut vegetables, sea foods and sprouts (Ding et al., 2015; Issa-Zacharia et al., 2011; Rui et al., 2011). Our results also demonstrated the disinfection efficacy of SAEW in GBM during germination. Due to its dual effects of microbial decontamination and the improvement of GABA accumulation, SAEW showed a promising prospect in the production of GBM.

5. Conclusions

In this work, the effect of SAEW treatment on the accumulation of GABA in GBM was investigated during germination. The results showed that SAEW treatment with appropriate ACC (15 or 30 mg/L) could promote the accumulation of GABA by up to 21%.

However, the morphological measurements showed that SAEW treatment could not promote the sprouts growth of GBM in comparison with TW treatment. Our results suggested that the GABA accumulation were independent of the length of sprouts in germinated grains. In addition, SAEW treatment reduced the microbial populations in millet dipped for 2 h by 0.76–1.07 log₁₀CFU g⁻¹ and resulted in the reduction of 0.7–1.11 log₁₀CFU g⁻¹ during millet germination. SAEW treatment with high ACC (15 and 30 mg/L) showed stronger anti-infection potential in GBM than that of lower ACC (5 mg/L). In conclusion, SAEW treatment avoiding too long millet sprouts is an available approach to improve the accumulation of GABA and anti-infection potential in GBM. Therefore, SAEW shows a promising prospect in the production of millet sprouts.

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