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Accumulation Mechanism of γ-Aminobutyric Acid in Tomatoes (*Solanum lycopersicum* L.) under Low O_2 with and without CO_2

Nobukazu Mae,[†] Yoshio Makino,*[,†] Seiichi Oshita,[†] Yoshinori Kawagoe,[†] Atsushi Tanaka,[‡,⊗] Koh Aoki,[§,∥] Atsushi Kurabayashi,[∥] Takashi Akihiro,[⊥] Kazuhito Akama,[⊥] Satoshi Koike,[#] Mariko Takayama,[#] Chiaki Matsukura,[#] and Hiroshi Ezura[#]

[†]Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan

[‡]P-Plus Project, Sumitomo Bakelite Company Limited, 495, Akiba-cho, Totsuka-ku, Yokohama 245-0052, Japan

[§]Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Nakaku, Sakai, Osaka 599-8531, Japan

[∥]Laboratory of Bioengineering, Kazusa DNA Research Institute, 2-6-7, Kazusa-Kamatari, Kisarazu, Chiba 292-0818, Japan

[⊥]Faculty of Life and Environmental Science, Shimane University, 1060 Nishikawatsu-cho, Matsue, Shimane 690-8504, Japan

[#]Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1, Tennodai, Tsukuba, Ibaraki 305-8572, Japan

ABSTRACT: The storage of ripe tomatoes in low-O_2 conditions with and without CO_2 promotes γ-aminobutyric acid (GABA) accumulation. The activities of glutamate decarboxylase (GAD) and α-ketoglutarate-dependent GABA transaminase (GABA-TK) were higher and lower, respectively, following storage under hypoxic (2.4 or 3.5% O_2) or adjusted aerobic (11% O_2) conditions compared to the activities in air for 7 days at 25 °C. GAD activity was consistent with the expression level of mRNA for GAD. The GABA concentration in tomatoes stored under hypoxic conditions and adjusted aerobic conditions was 60−90% higher than that when they are stored in air on the same day. These results demonstrate that upregulation of GAD activity and downregulation of GABA-TK activity cause GABA accumulation in tomatoes stored under low-O_2 conditions. Meanwhile, the effect of CO_2 on GABA accumulation is probably minimal.

KEYWORDS: *amino acid, enzymatic activity, fruits, hypoxia, modified atmosphere packaging, mRNA*

INTRODUCTION

The functional compound γ-aminobutyric acid (GABA), known for its relaxing effects in humans[1] and antihypertensive effect,[2] is widely distributed throughout the biological world.[3] Although GABA is present in plant tissue at low levels as well, it increases by several folds in response to diverse stimuli, such as heat shock, mechanical stimulation, hypoxia, and phytohormones.[4]

The accumulation of GABA as an important functional compound has been attempted in several food types, including rice (*Oryza sativa* L.) kernel,[5] tea [*Camellia sinensis* (L.) O. Kuntze],[6] and soybean [*Glycine max* (L.) Merrill].[7]

Because GABA is relatively abundant in tomatoes (*Solanum lycopersicum* L.), Saito et al.[8] screened GABA-rich tomato cv. 'DG03-9'. Stress from anoxia is reported to effectively stimulate GABA production;[3] however, horticultural products stored under anoxic or hypoxic conditions generate off-flavors, thereby deteriorating their commercial value.[9] Makino et al.[10] reported that adjusted aerobic as well as hypoxic conditions were effective for stimulating GABA production in tomatoes at 30 °C. Mae et al.[11] reported that adjusted aerobic conditions of 11% O_2 and 12% CO_2 were effective in stimulating GABA accumulation in tomatoes at 25 °C. Deewatthanawong et al.[12] reported that air containing 10% CO_2 was also effective in stimulating GABA accumulation in tomatoes. Although the stimulation of GABA accumulation in tomatoes stored under low-O_2 atmospheres is a well-known phenomenon, the physiological mechanism remains unclear.

In the present study, we aimed to elucidate the physiological mechanism of GABA accumulation in tomatoes stored in low-O_2 conditions with and without CO_2. Tomatoes were stored under five different atmospheric conditions that were controlled using different packaging materials, and changes in concentrations of metabolites, enzymatic activities associated with GABA shunt, and the messenger ribonucleic acid (mRNA) for glutamate decarboxylase (GAD) were measured. Here, we discuss the relationship between the measured data and atmospheric conditions to clarify the mechanism of GABA accumulation under low-O_2 conditions with and without CO_2.

MATERIALS AND METHODS

Materials. Ripe tomatoes (*S. lycopersicum* L., Momotaro cultivar) harvested on June 30, 2010, from an orchard in Wakayama Prefecture, Japan, were sampled. After the harvest, the samples were kept at 10−16 °C, transported to the laboratory within approximately 24 h, and stored at a constant temperature unit (15 °C) for another 24 h before experimentation. A total of 96 intact tomatoes (mean, 193 g; range, 166−231 g) were used without any pretreatments for the following experiments.

Methods. Two tomatoes were sealed in pouches under five different atmospheric conditions (Table 1). A total of 45 pouches (nine pouches

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Table 1. Packaging Conditions Tested in the Present Study[a]

atmosphere	pouch number	CO_2 absorbent	C_2H_4 absorbent
hypoxic with CO_2	1	−	+
hypoxic without CO_2	1	+	+
adjusted aerobic with CO_2	2	−	+
adjusted aerobic without CO_2	3	+	+
air	4	−	+

[a]Two tomatoes were sealed in a pouch. The masses of both CO_2 and C_2H_4 absorbents were 10 g. Pouch numbers: 1, three-layer laminated [polyethylene terephthalate (PET)/aluminum/polyethlene] high-barrier pouch Lamizip (AS ONE Corporation, Tokyo, Japan) (O_2 permeance, 1.7×10^{-5} mmol m^{-2} h^{-1} kPa^{-1}; film thickness, 110 μm; surface area, 0.094 m^2); 2, microperforated PET/low-density polyethylene (LDPE) film pouch (O_2 permeance, 0.13 mmol m^{-2} h^{-1} kPa^{-1}; film thickness, 47 μm; surface area, 0.085 m^2); 3, microperforated PET/LDPE film pouch (O_2 permeance, 0.22 mmol m^{-2} h^{-1} kPa^{-1}; film thickness, 47 μm; surface area, 0.085 m^2); and 4, macroperforated pouch (the same PET/LDPE film as the microperforated pouch 3, with 6 mm inner diameter × 8 holes).

for each condition) were prepared and stored at 25 °C for 7 days in a dark, constant temperature unit. The storage temperatures and periods were selected to stimulate the enzymatic reactions associated with GABA production according to the method described by Mae et al.[11] During storage, the in-package atmospheric conditions (O_2, CO_2, and C_2H_4 concentrations) were analyzed by gas chromatography, as previously described;[13−15] tomatoes were sampled 3 times. Therefore, three pouches from each condition were selected and subjected to chemical analysis. The sampled tomatoes were stored at −80 °C until the measurement of metabolic compounds, mRNA accumulation, and GAD activity. Raw samples were used for the measurement of all enzymatic activities, except those of GAD.

Samples for the analysis of metabolic compounds associated with GABA shunt in tomatoes (Table 2) were prepared according to the method reported by Akihiro et al.[16] All compounds, except succinic semialdehyde, were measured using the GC 6890 (Agilent Technologies, Santa Clara, CA) by the method described by Akihiro et al.[16] Succinic semialdehyde was measured using the GABase assay reported by Streeter and Thompson.[17] For the measurement mentioned above, two tomatoes from each pouch were mixed (n = 3 at each measurement).

The activities of enzymes associated with GABA shunt were measured according to the method reported by Akihiro et al.[16] The samples stored at −80 °C were used for the measurement of GAD activity. The raw samples were used for measuring α-ketoglutarate-dependent GABA transaminase (GABA-TK), pyruvate-dependent GABA transaminase (GABA-TP), and succinic semialdehyde dehydrogenase (SSADH) activities. For the measurement of the activities, six fruits in three pouches were mixed (n = 1 measurement) because the value should be measured very rapid before the inactivation of enzymes occurs.

Transcriptional levels of *SlGAD1*, *SlGAD2*, and *SlGAD3* (accession numbers AB359913, AB359914, and AB359915) were estimated by the following method. For RNA extraction, 50 mg of frozen pericarp tissues were ground with a mortar and pestle. Total RNA was prepared using the RNeasy Plant Minikit (Qiagen, Valencia, CA). Total RNA (1.5 μg) was subjected to reverse transcription (RT) as a template for each sample and converted to double-stranded cDNA using the SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA) according to the instructions of the manufacturer. Quantitative RT-polymerase chain reaction (PCR) was performed by the SYBR Green detection method using the Takara Thermal Cycler Dice Real Time System TP800 with SYBR Premix Ex Taq (Takara BioInc., Otsu, Japan). The reaction cycles were as follows: 95 °C for 10 s for initial denaturation, followed by 40 cycles of 5 s of denaturation at 95 °C, and 30 s of annealing/extension at 54 °C. The gene-specific primers for *SlGAD1*, *SlGAD2*, and *SlGAD3* were previously described by Akihiro et al.[16] The tomato ubiquitin gene (*SlUBI3*) (accession number X58253) was used as an internal standard. Transcriptional levels of the target genes were calculated in relation to the transcriptional level of *SlUBI3* according to Kim et al.[18] Primer sequences were as follows: *SlGAD1* forward, 5′-AAACTTCCCATTTCCCAACC-3′, reverse, 5′-CGATTGATCGGAGGAGAAAA-3′; *SlGAD2* forward, 5′-CTTTGATCTTCTCCGTCGTTG-3′, reverse, 5′-ATATCGAGACGCGAAAGTCG-3′; *SlGAD3* forward, 5′-CAGGACGTTTCAATATAATC-3′, reverse, 5′-CCTACGGAGGGTCTCAGAG-3′; and *SlUBI3* forward, 5′-CACCAAGCCAAAGAAGATCA-3′, reverse, 5′-TCAGCATTAGGGCACTCCTT-3′.

JMP 8.0.2 software (SAS Institute, Inc., Cary, NC) was used for statistical analysis. When between-class variation was significant at $p < 0.05$ using one-way ANOVA of the data, mean values were compared by the LSD test ($p < 0.05$).

RESULTS AND DISCUSSION

Gas composition in the macroperforated pouch was the same as that in the surrounding air. Changes in O_2 and CO_2 concentrations in the high-barrier and microperforated film pouches over time are shown in Figure 1. In the high-barrier

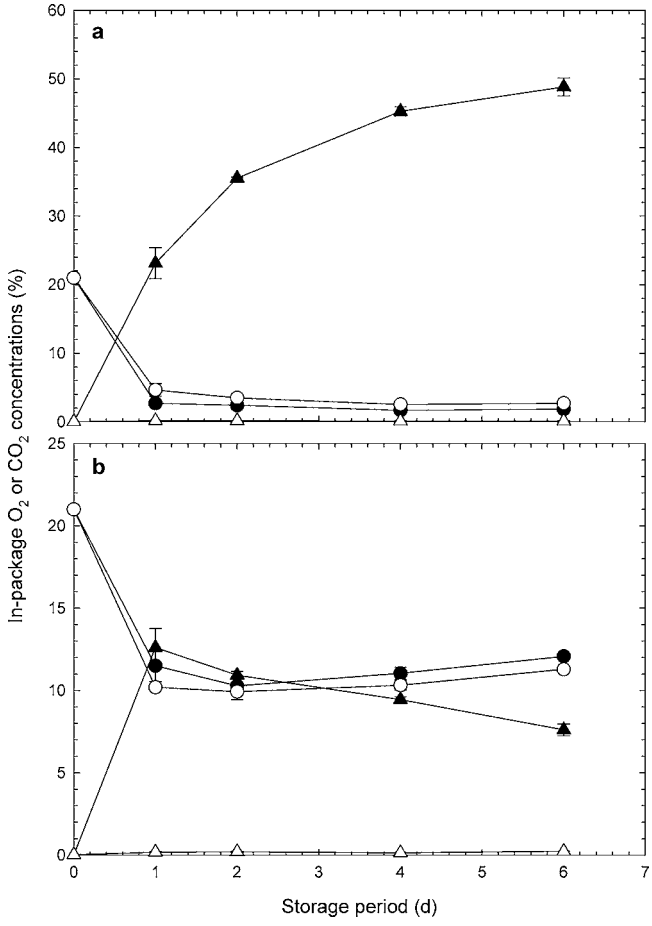

Figure 1. In-package atmospheric changes in (a) three-layer laminated high-barrier and (b) microperforated film pouches at 25 °C (n = 3). O_2 and CO_2 concentrations in macroperforated film pouches were 21 and 0% throughout the storage period, respectively. Circles and triangles denote in-package O_2 and CO_2 concentrations, respectively. Open and closed symbols denote the atmospheres with and without CO_2 absorbent, respectively. The mean ± SE of three observations has been plotted. In-package O_2 and CO_2 concentrations were significantly different ($p < 0.05$; one-way ANOVA with LSD test) among pouches from the same time points.

pouch, the mean O_2 concentration decreased from 21 to 2.4 or 3.5% after 1 day of storage and remained at approximately 2%

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Table 2. Changes in Concentrations of Metabolic Compounds Associated with GABA Shunt (μmol 100 g^{-1} of fresh weight)[a]

compound	atmosphere	storage days 0	3	5	7
	hypoxia with CO_2		264 ± 11.3	184 ± 28.9 b	287 ± 39.4 b
	hypoxia without CO_2		422 ± 61.7	419 ± 96.7 a	333 ± 20.6 b
alanine	adjusted aerobic with CO_2	239 ± 55.6	408 ± 63.8	492 ± 43.7 a	585 ± 47.1 a
	adjusted aerobic without CO_2		298 ± 110	436 ± 34.0 a	628 ± 123 a
	air		177 ± 43.1	211 ± 23.3 b	202 ± 34.4 b
	hypoxia with CO_2		499 ± 21.0	465 ± 36.4	523 ± 24.7 b
	hypoxia without CO_2		540 ± 84.0	584 ± 66.8	525 ± 28.4 b
asparatic acid	adjusted aerobic with CO_2	517 ± 36.2	495 ± 56.9	525 ± 25.7	529 ± 26.6 b
	adjusted aerobic without CO_2		549 ± 95.2	585 ± 67.4	487 ± 30.4 b
	air		564 ± 30.2	634 ± 15.9	722 ± 41.6 a
	hypoxia with CO_2		30400 ± 1820	28800 ± 1180	31800 ± 1670
	hypoxia without CO_2		35600 ± 6150	29500 ± 3540	27100 ± 2160
citrate	adjusted aerobic with CO_2	35300 ± 4610	26100 ± 3790	29400 ± 4120	31900 ± 3350
	adjusted aerobic without CO_2		30500 ± 5350	28300 ± 850	30700 ± 2300
	air		28500 ± 3150	30000 ± 636	24500 ± 2510
	hypoxia with CO_2		1.41 ± 0.167	2.21 ± 0.362	3.01 ± 0.309
	hypoxia without CO_2		1.67 ± 0.193	1.86 ± 0.328	3.52 ± 0.0833
fumarate	adjusted aerobic with CO_2	1.34 ± 0.119	1.37 ± 0.0883	1.54 ± 0.127	2.83 ± 0.678
	adjusted aerobic without CO_2		1.66 ± 0.0390	1.63 ± 0.465	3.08 ± 0.463
	air		1.30 ± 0.182	2.01 ± 0.0584	3.12 ± 0.592
	hypoxia with CO_2		816 ± 60.4	1000 ± 13.5 a	1150 ± 45.7 a
	hypoxia without CO_2		1110 ± 205	949 ± 407 ab	963 ± 44.7 a
GABA	adjusted aerobic with CO_2	814 ± 71.7	618 ± 212	697 ± 58.2 bc	1060 ± 137 a
	adjusted aerobic without CO_2		777 ± 111	666 ± 98.6 c	979 ± 121 a
	air		535 ± 99.6	691 ± 23.7 bc	601 ± 57.8 b
	hypoxia with CO_2		478 ± 52.7	404 ± 16.7 c	544 ± 53.3
	hypoxia without CO_2		708 ± 172	523 ± 85.5 bc	535 ± 56.0
glutamate	adjusted aerobic with CO_2	402 ± 13.0	460 ± 84.7	556 ± 12.4 ab	566 ± 52.2
	adjusted aerobic without CO_2		525 ± 98.9	521 ± 18.8 b	634 ± 27.9
	air		547 ± 28.6	660 ± 25.4 a	658 ± 40.6
	hypoxia with CO_2		98.0 ± 12.2	78.7 ± 5.79	73.9 ± 2.02
	hypoxia without CO_2		107 ± 7.97	72.3 ± 3.78	75.9 ± 6.73
isocitrate	adjusted aerobic with CO_2	122 ± 9.77	111 ± 14.9	63.4 ± 1.40	80.3 ± 10.4
	adjusted aerobic without CO_2		96.2 ± 20.1	73.0 ± 7.50	84.9 ± 8.00
	air		81.6 ± 2.83	84.8 ± 1.92	68.7 ± 7.15
	hypoxia with CO_2		261 ± 12.5	223 ± 21.0	211 ± 11.7
	hypoxia without CO_2		224 ± 35.4	222 ± 21.7	177 ± 13.3
malate	adjusted aerobic with CO_2	327 ± 41.6	193 ± 2.57	184 ± 8.86	196 ± 11.7
	adjusted aerobic without CO_2		215 ± 18.3	186 ± 43.0	178 ± 24.3
	air		175 ± 11.0	173 ± 8.67	140 ± 9.27
	hypoxia with CO_2		9.14 ± 1.50	26.9 ± 1.69 a	26.3 ± 2.12 a
	hypoxia without CO_2		7.56 ± 3.76	11.9 ± 2.73 b	20.3 ± 4.44 ab
succinate	adjusted aerobic with CO_2	3.61 ± 0.648	3.48 ± 0.447	1.86 ± 0.970 c	11.0 ± 4.18 bc
	adjusted aerobic without CO_2		0.981 ± 0.0999	1.49 ± 0.881 c	10.3 ± 7.85 bc
	air		3.44 ± 1.02	1.93 ± 0.961 c	1.96 ± 0.120 c
	hypoxia with CO_2		21.7 ± 4.72	25.1 ± 3.34	36.6 ± 3.63
	hypoxia without CO_2		32.0 ± 8.97	28.7 ± 4.60	34.5 ± 6.53
succinic semialdehyde	adjusted aerobic with CO_2	34.8 ± 2.48	24.5 ± 9.68	25.5 ± 9.20	31.9 ± 6.80
	adjusted aerobic without CO_2		32.6 ± 9.58	32.6 ± 3.73	20.8 ± 3.85
	air		34.5 ± 2.08	24.2 ± 4.97	30.3 ± 8.00

[a]Values are means ± standard error (SE) of three observations. Within the same compound and storage day, symbols followed by the same letter are not significantly different [$p < 0.05$; one-way analysis of variance (ANOVA) with least significant difference (LSD) test].

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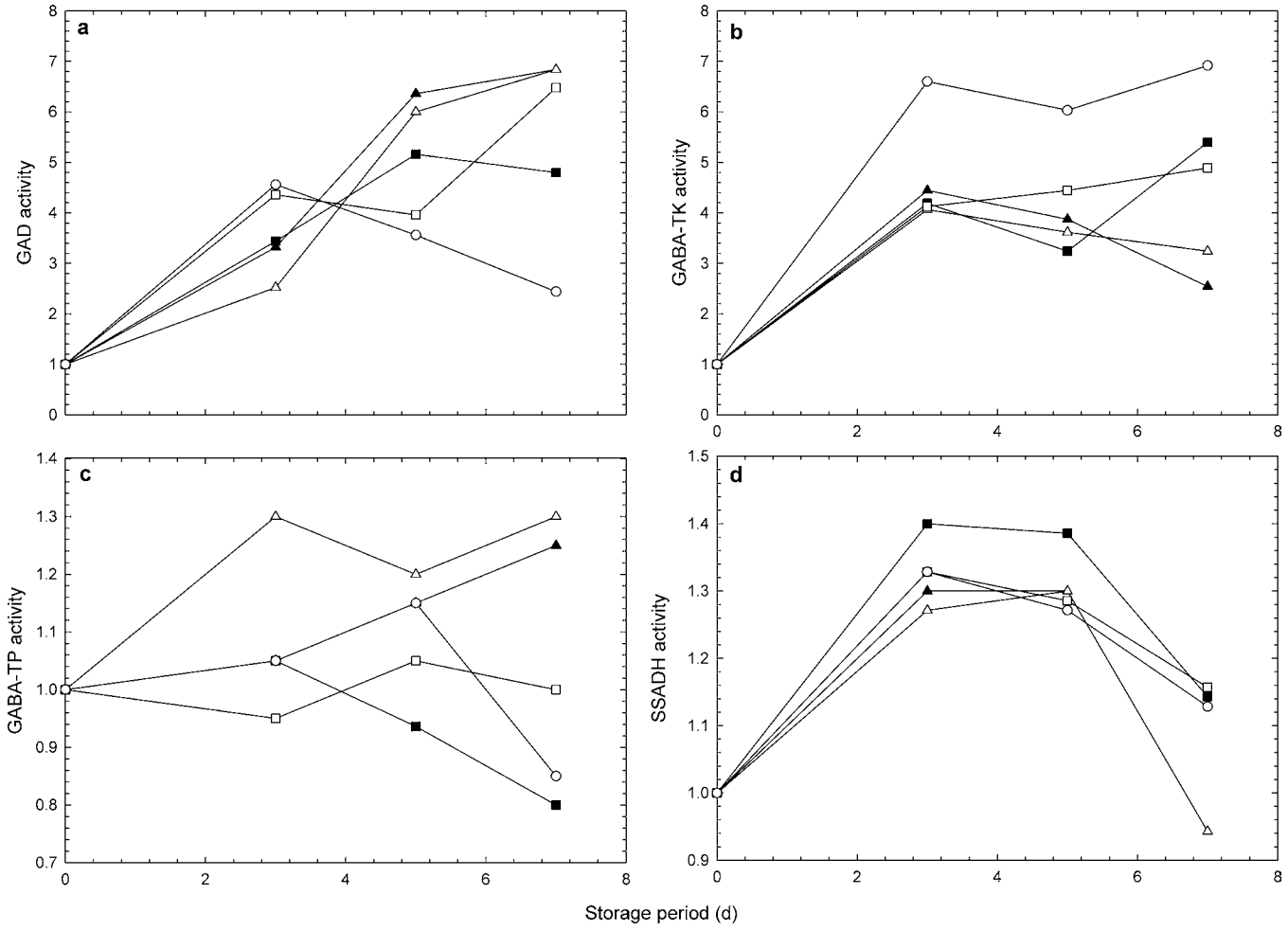

Figure 2. Changes in (a) GAD-, (b) GABA-TK-, (c) GABA-TP-, and (d) SSADH-specific activities under different atmospheres. Triangles, squares, and circles denote the values under hypoxic conditions, under adjusted aerobic atmospheric conditions, and in air, respectively. Solid and open symbols denote the values in atmospheric conditions with and without CO_2, respectively.

until the end of gas analysis (6 days). The mean CO_2 concentration in a pouch without CO_2 absorbent increased from 0 to 23% after 1 day and gradually increased to 49% by the end of gas analysis. In a pouch with CO_2 absorbent, the CO_2 concentration was maintained at near 0%. In the microperforated film pouches, a steady state was reached after 1 day of storage. A mean O_2 of approximately 11% was maintained until the end of gas analysis. Mean CO_2 concentrations of approximately 10 and 0% were maintained in a pouch with and without CO_2 absorbent, respectively. The gas composition in the storage pouches was hypoxic in the high-barrier pouch and aerobic in the microperforated film pouch.[19] Ethylene (C_2H_4) concentrations were maintained at 0 ppm in all pouches, and therefore, in-package ethylene did not affect the experimental data. The in-package atmospheres created in the present study were feasible for observing the effect of atmospheric conditions on dynamic changes in metabolites and enzymatic activities associated with the GABA shunt.

Changes in the concentrations of metabolites associated with GABA shunt over time are presented in Table 2. The decrease in O_2 affected changes in alanine, asparatic acid, GABA, glutamate, and succinate among the measured metabolites. Meanwhile, increases in CO_2 had little influence on the changes in metabolites. The GABA concentration was significantly higher under hypoxic conditions than under aerobic conditions after 5 days of storage, and it was significantly lower in air than under other atmospheric conditions at the end of storage (7 days). These results agree with those of the previous study performed at 25 °C[11] and support the reproducibility of the observations in the present study. CO_2 did not cause the difference in the GABA concentration. Deewatthanawong et al.[12] reported that the GABA concentration in the breaker stage and red tomatoes increased during storage for 12 days at 13 °C under 10% CO_2 conditions. Makino et al.[10] reported that GABA concentrations in vine ripe tomatoes did not significantly increase during storage for 13 days at 15 °C under approximately 17% CO_2 with approximately 5% O_2 or 8% CO_2 with approximately 12% O_2, although the GABA concentrations were higher than those in tomatoes stored in air. Although CO_2 treatment may stimulate GABA production depending upon storage conditions or the state of the samples, a decrease in the O_2 level may be more effective in stimulating GABA production than increasing CO_2, according to the results in Table 2. The alanine concentrations of tomatoes stored under the adjusted aerobic atmospheric conditions were significantly higher than those under the other atmospheric conditions at 7 days of storage. In several published studies,[10,11] alanine production was stimulated under adjusted aerobic atmospheric conditions. The same phenomenon repeatedly occurred in the present study as well. Alanine and GABA production in plant tissue has been known to be stimulated by similar conditions since the 1970s.[17] Alanine concentrations are believed

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to be higher under the adjusted aerobic conditions than under hypoxic conditions because the strong O_2 stress promotes the decomposition of accumulated alanine by alanine aminotransferase.[20]

The glutamate concentrations of tomatoes stored in air were significantly higher than those under hypoxic conditions after 5 days of storage. However, no difference was observed in the concentrations at the end of storage (7 days). This compound is converted into GABA by the GAD reaction and is produced by the GABA-TK reaction. Therefore, the glutamate concentration may be associated with the reactions of these two enzymes.

Similar to GABA concentrations, the succinate concentrations of tomatoes stored under hypoxic conditions were significantly higher than those under the other atmospheric conditions after 5 days of storage and those in air by the end of storage (7 days). This result suggests that GABA accumulation affects the succinate concentration. Meanwhile, the concentration of succinic semialdehyde as an intermediate product of the enzymatic reaction to produce succinate from GABA and the concentration of fumarate as a product of succinate were not affected by atmospheric conditions. Akihiro et al.[16] reported that the succinate concentration in a GABA-rich tomato cultivar was significantly higher than that in a normal cultivar at the immature green stage. However, the opposite result occurred at the red stage. These results were partially not consistent with the results in the present study. This suggests that the mechanism of stimulation in GABA production by the decrease in O_2 is not the same as that in the GABA-rich cultivar. The aspartic acid concentration in tomatoes stored in air was significantly lower than that under the other atmospheric conditions at the end of storage (7 days). This result agreed with that of the study conducted using the GABA-rich cultivar.[16] Aspartic acid and GABA are produced from glutamate. Therefore, its production may be suppressed when the GABA production is stimulated.

Changes in enzymatic activities associated with GABA shunt over time are presented in Figure 2.

The GAD activities of tomatoes stored under hypoxic and adjusted aerobic atmospheric conditions were higher than those of tomatoes stored in air after 5 days of storage. Because GAD is the enzyme that produces GABA, an increase in GAD activity stimulated GABA production. According to a report by Akihiro et al.,[16] GAD activity in tomatoes (cv. Micro-Tom and DG03-9) did not increase after the breaker stage. However, the tomatoes were not stored under hypoxic or adjusted aerobic atmospheric conditions in their study. Deewatthanawong et al.[12] reported that GAD activity in tomatoes at the red stage was higher under 10% CO_2 conditions than in air between 6 and 15 days of storage. In the present study, the effect of CO_2 on the increase of GAD activity was not observed. Therefore, the increase was caused by the decrease in the O_2 concentration.

The GABA-TK activities of tomatoes stored under the hypoxic and adjusted aerobic atmospheric conditions were lower than those in air after 5 days of storage. Because GABA-TK catalyzes the reaction of GABA and α-ketoglutarate to glutamate and succinic semialdehyde, the decrease in GABA-TK activity caused GABA accumulation. Akihiro et al.[16] reported that GABA-TK activity was associated with GABA accumulation in tomatoes. The GABA-TK activities of tomatoes stored under hypoxic conditions were lower than those under the adjusted aerobic atmospheric conditions after 7 days of storage. This suggests that a decrease in O_2 effectively decreases GABA-TK activity. In the present study, a decrease in GABA-TK activity by CO_2 was unclear.

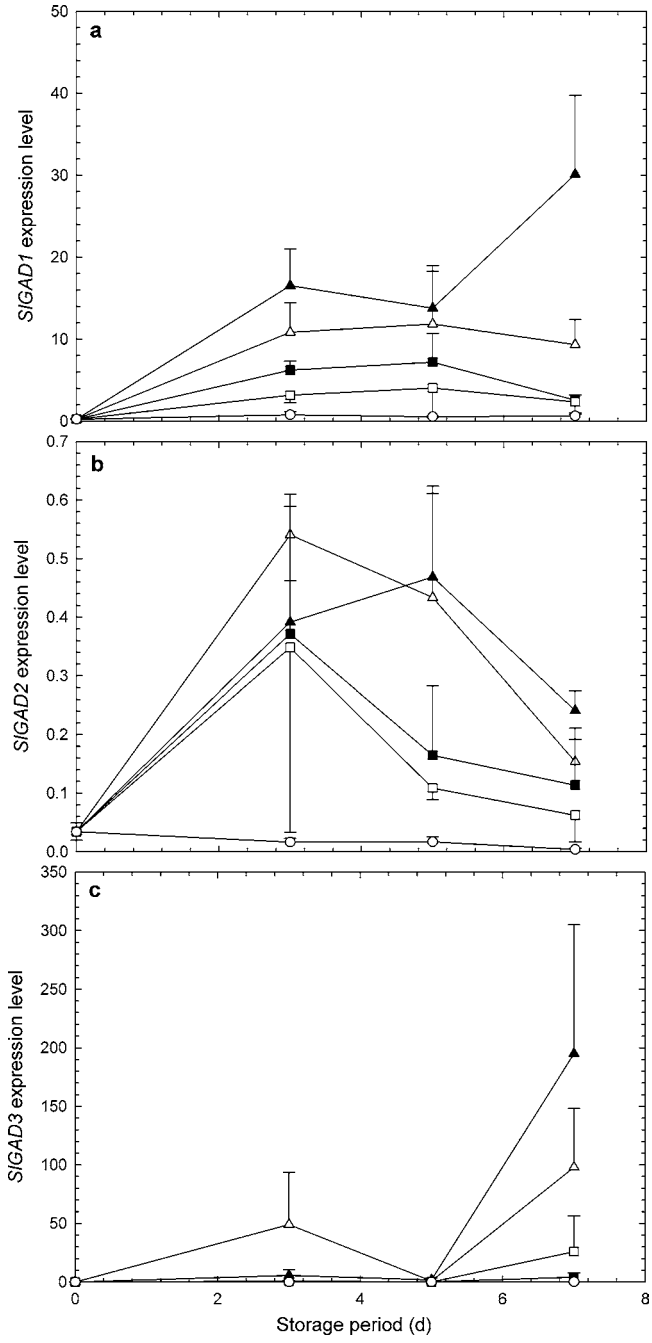

Figure 3. Changes in mRNA expression of (a) *SlGAD1*, (b) *SlGAD2*, and (c) *SlGAD3* genes in tomatoes stored under different atmospheres. Triangles, squares, and circles denote the values under hypoxic conditions, under adjusted aerobic conditions, and in air, respectively. Solid and open symbols denote the values in atmospheric conditions with and without CO_2, respectively. The relative quantification of GAD gene expression was calculated using the tomato *SlUBI3* gene (accession number X58253) as an internal control. The mean ± SE of three observations has been presented.

The GABA-TP activities of tomatoes stored under hypoxic conditions were higher than those under other atmospheric conditions after 7 days of storage. Because GABA-TP is the enzyme that catalyzes the reaction of GABA and pyruvate to alanine and succinic semialdehyde, an increase in GABA-TP activity causes the stimulation of GABA decomposition. However, the GABA concentration in tomatoes stored under hypoxic conditions was higher than that in tomatoes stored in

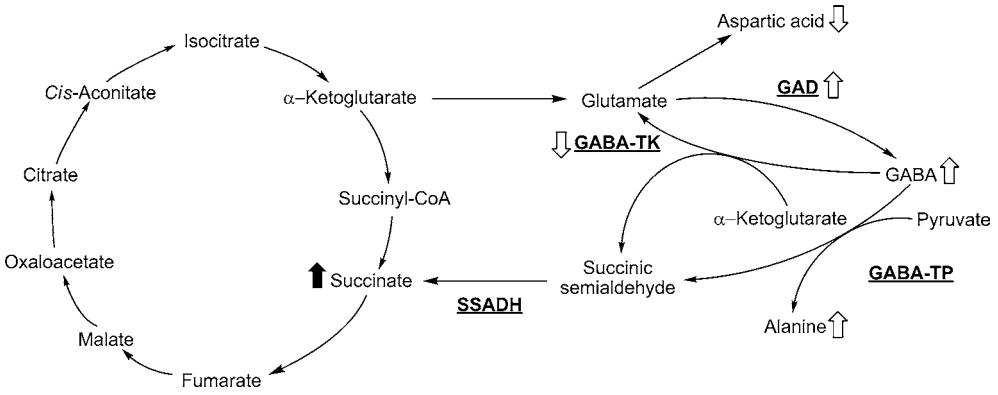

Figure 4. Summary of the effects of the O_2 decrease on dynamic changes in compounds and enzymatic activities associated with GABA shunt. Up- and down-pointing arrows denote the compounds, and enzymatic activities (GAD, GABA-TK, GABA-TP, and SSADH) significantly increased and decreased with the decrease in O_2, respectively. Solid arrows denote the compounds that significantly increased under hypoxic conditions.

air after 5 days of storage. Therefore, GABA-TP activity may not have affected the GABA concentration. This result agreed with the results reported by Akihiro et al.[16] Clark et al.[21] also examined the activity of GABA-TP.

The SSADH activities of tomatoes stored under hypoxic conditions were lower than those of tomatoes stored under the other atmospheric conditions after 7 days of storage. SSADH is the enzyme that catalyzes the reaction from succinic semialdehyde to succinate. Deewatthanawong et al.[12] reported that SSADH activity in tomatoes at the red stage was lower under the conditions with 10% CO_2 than that in air on 12 days of storage. However, they did not discuss the relationship between the SSADH activity and the GABA concentration as thoroughly as Akihiro et al.[16]

The changes in the expression levels of mRNA for GAD over time are presented in Figure 3. According to the mean values, the expression levels were higher with the decrease in O_2 concentrations. Akihiro et al.[16] reported that the GABA concentrations in tomatoes were in good agreement with the levels of *SlGAD2* and *SlGAD3* gene expressions. Therefore, GABA production may be stimulated by an increase in the expression levels of mRNA for GAD by the decrease in O_2. Deewatthanawong et al.[12] reported that the relative expressions of *GAD2* and *GAD3* were increased in CO_2-treated fruit. However, whether CO_2 affected the expression of mRNA for GAD in the present study remained unclear.

A summary of the effects of the O_2 decrease on dynamic changes in metabolites and enzymatic activities associated with GABA shunt is shown in Figure 4. In conclusion, increases in GAD activity and decreases in GABA-TK activity caused GABA accumulation in tomatoes and the concentrations of a few associated compounds were affected by the changes in enzymatic activities. In the present study, GABA accumulation occurred with an increase in GAD activity under low-O_2 atmospheric conditions but GAD activity was not as significantly affected by CO_2 levels.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +81-358415361. Fax: +81-358418174. E-mail: amakino@mail.ecc.u-tokyo.ac.jp.

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Present Address

⊗P-Plus Project, Sumitomo Bakelite Co., Ltd., 2-3-47 Higashitsukaguchi-cho, Amagasaki, Hyogo 661-0011, Japan.

ABBREVIATIONS USED

ANOVA, analysis of variance; GABA, γ-aminobutyric acid; GAD, glutamate decarboxylase; GABA-TK, α-ketoglutarate-dependent GABA transaminase; GABA-TP, pyruvate-dependent GABA transaminase; SSADH, succinic semialdehyde dehydrogenase; LSD, least significant difference; mRNA, messenger ribonucleic acid; SE, standard error

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