Original Paper

Stimulation of γ-Aminobutyric Acid Production in Vine-ripened Tomato (*Solanum lycopersicum* L.) Fruits Under an Adjusted Aerobic Atmosphere

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Stimulation of γ-aminobutyric acid (GABA) production under low O_2 and high CO_2 conditions (adjusted aerobic atmosphere) at two levels of temperature was studied. Vine-ripened tomato fruits were stored under hypoxic conditions and adjusted aerobic atmospheres as well as in air at 15°C for 12.6 d and at 25°C for 6.6 d. At 15°C, the GABA concentrations of tomato fruits during storage under one adjusted aerobic atmosphere (O_2 14%, CO_2 8%) were not significantly different from that in air. On the other hand, at 25°C, the tomato fruit GABA concentrations under a second adjusted aerobic atmosphere (O_2 11%, CO_2 12%) were significantly higher by 72% than that in air 6.6 d from the start of storage. Increased accumulation of alanine under this adjusted aerobic atmosphere supports the observation that these conditions stimulate GABA production. This temperature is 5°C lower than the temperature (30°C) reported to be required to stimulate GABA production. This result is more significant than that reported in the past because the shelf-life of fresh produce including tomato fruits can be prolonged by storage at low temperatures.

Keywords : *Solanum lycopersicum* L., γ-aminobutyric acid, modified atmosphere packaging

1. Introduction

The functional compound γ-aminobutyric acid (GABA)[1-3] is widely distributed throughout the biological world[4]. Accumulation of GABA in horticultural products has been well studied. Akihiro et al.[5] found that a high GABA concentration in a variety of tomato fruits was caused by low enzymatic activity of α-ketoglutarate-dependent GABA transaminase.

Previous reports have shown that GABA accumulates rapidly in plant tissues exposed to a variety of different stresses[4]. Stress from anoxia is reported to be effective for stimulating GABA production[4]; however, horticultural products stored under anoxic or hypoxic conditions generate off-odors due to ethanol fermentation and lose their commercial value[6]. Makino et al.[7] reported that an adjusted aerobic atmosphere is also effective for stimulating GABA production

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in tomato fruits at 30°C as well as under hypoxic conditions. However, this temperature is rather high for the storage of fresh produce. Furthermore, Gautier et al.[8] reported suppression of normal ripening when the temperature was increased from 27 to 32°C in experiments using mature green tomato fruits. Therefore, low storage temperature and conditions that stimulate GABA production are required. In the present study, we attempted to stimulate GABA production in tomato fruits at 25°C to reduce storage temperature by 5°C than the previous study[7] and compared this stimulation with that in fruits stored at 15°C.

2. Materials and Methods

2.1 Materials

Vine-ripened tomato fruits (*Solanum lycopersicum* L.[9], House Momotaro cultivar) were harvested on November 15 and December 13, 2007, at a farm in Hiratsuka (Kanagawa Prefecture, Japan). After harvesting, the samples were maintained at 10–16°C, transported to the laboratory within 1 d, and stored in a constant temperature unit (15°C) for another 1 d before use. The average mass of the 78 pieces of fruit harvested on November 15 was 0.198 kg (S.D. ± 0.012; range 0.175–0.225 kg). The average mass of the 78 pieces of fruit harvested on December 13 was 0.196 kg (S.D. ± 0.012; range 0.171–0.226 kg). Intact fruits without any pretreatments were used for the following experiments.

Three-layer laminated (polyethylene terephthalate (PET)/aluminum/polyethlene) high barrier pouches Lamizip® (AS ONE Corporation, Tokyo) (O_2 permeance: 1.7×10^{-5} mmol·m^{-2}·h^{-1}·kPa^{-1}, film thickness: 110 μm; surface area: 0.094 m^2) were used to create hypoxic conditions with high CO_2 levels around the tomato fruits. Micro-perforated PET/low-density polyethylene (LDPE) film pouches (O_2 permeance: 0.16 mmol·m^{-2}·h^{-1}·kPa^{-1}, film thickness: 47 μm; surface area: 0.085 m^2) were used to contain a controlled aerobic atmosphere at 15 and 25°C, respectively. The atmospheric conditions were created as previously described[10]. Macro-perforated pouches (the same PET/LDPE film as the micro-perforated pouch with 5 mm∅ × 8 holes) were used as a control in which the in-package atmosphere was expected to be the same as that of ambient air.

2.2 Storage of Tomato Fruits

Two tomato fruits were sealed in a pouch containing 10 g C_2H_4 absorbent (Picón et al., 1993) using an impulse sealer. A total of 72 pouches with C_2H_4 absorbent were prepared and stored at 15°C for 12.6 d (harvested in November) and at 25°C for 6.6 d (harvested in December) in dark constant temperature units. The temperature of 15°C was the storage temperature effective for maintaining the quality of tomato fruits according to Nakhasi et al.[11]. In contrast, the storage temperature (25°C) was selected in an attempt to reduce the temperature at which GABA production is stimulated compared to that of 30°C selected in the previous study[7]. Storage periods at each temperature were subjectively determined and were terminated when the fruit lost its visual commercial value due to softening[12] or growth of fungi[13].

2.3 Data Analysis

Changes in the in-package O_2 and CO_2 concentrations were monitored using the method

of Makino et al.[14].

Changes in amino acid concentrations of all tomato samples were monitored using the method of Makino et al.[7].

JMP® 8.0 software (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. When between-class variation was significant at $P < 0.05$ using one-way analysis of variance (ANOVA), mean values were compared by the least significant difference (LSD) test ($P < 0.05$).

3. Results and Discussion

Gas composition in the macro-perforated pouch was the same as that in the surrounding air regardless of temperature. Changes in O_2 and CO_2 concentrations in the high barrier and micro-perforated pouches over time are shown in Fig.1. In the high barrier pouch at 15°C, the mean O_2 concentration decreased from approximately 21% to 5% after 4-d storage, and remained at that level until the end of storage (12.6 d). The mean CO_2 concentration increased from 0% to 13% after 2.2-d storage, and gradually increased to 16% at the end of storage. At 25°C, mean O_2 concentration decreased to 4% after 1.1-d storage and remained at approximately 2% until the end of storage (6.6 d). The CO_2 concentration increased to 18% after 1.1-d storage and continued to increase up to 41% at the end of storage. In the micro-perforated film pouches at 15°C, a steady state was reached 2.2 d from the start of storage. Mean O_2 and CO_2 concentrations of 14% and 8%, respectively, were maintained until the end of storage (12.6 d). At 25°C, a steady state was reached after 1.1-d

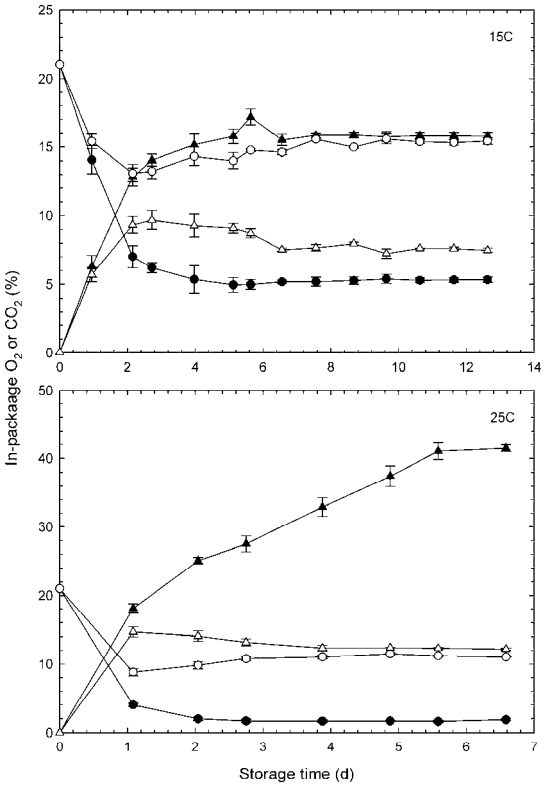

Fig.1 In-package atmosphere changes in pouches at 15 and 25℃ (n = 3). Open and closed symbols denote micro-perforated film and three-layer laminated high barrier pouches, respectively. Circles and triangles denote in-package O_2 and CO_2 concentrations, respectively. Mean ± SE of three observations are plotted.

storage, and mean O_2 and CO_2 concentrations of 11% and 12%, respectively, were maintained until the end of storage (6.6 d). The gas composition in the storage packaging was, regardless of storage temperatures, hypoxic in the high barrier pouch and aerobic in the micro-perforated film pouch.

Changes in GABA concentrations over time are shown in Fig.2. At 15°C, the GABA

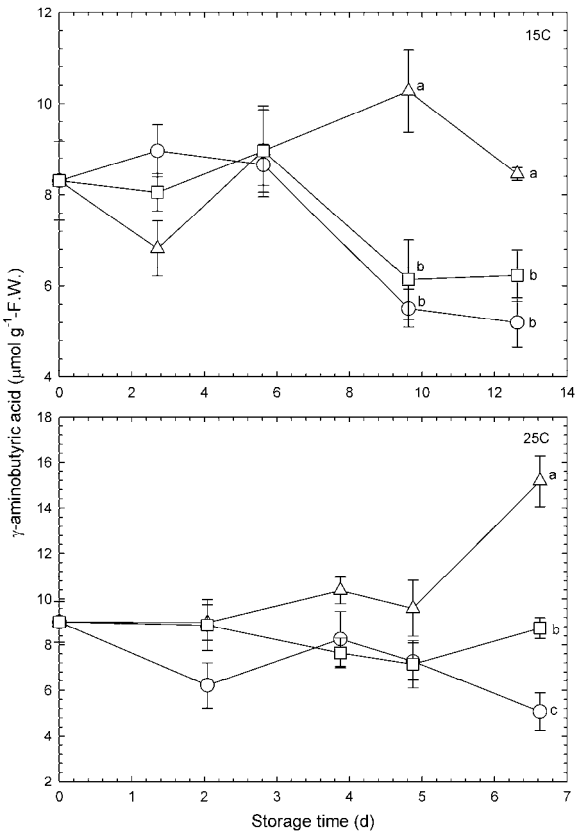

Fig.2 Changes in γ−aminobutyric acid in tomato fruits at 15 and 25℃ (n = 6). Circles, triangles, and squares denote values under ambient air, hypoxic conditions with high CO_2 levels, and adjusted aerobic (low O_2 and high CO_2 conditions) atmospheres, respectively. Mean ± SE of six observations are plotted. Within the same time interval, symbols followed by the same letter are not significantly different ($P < 0.05$; one−way ANOVA with LSD test).

concentration was significantly higher in the high barrier pouch than in the micro-and macro-perforated pouches after 9.6-d storage. According to a previous investigation[7], no significant differences were observed among the three packaging methods at 15°C. The difference between the two studies may be due to a difference in the variety of fruits. However, the observation that GABA in fruits stored under modified atmospheres was not significantly stimulated at 15°C was reproduced in the present study. These results suggest that hypoxic conditions are needed to stimulate GABA production at 15°C. However, hypoxic conditions causing off-odors due to fermentation are not desirable for the storage of fresh products. On the other hand, after 6.6-d storage at 25°C, the GABA concentrations were significantly higher in the micro-perforated pouches and in the high barrier pouches than that in the macro-perforated pouches. Decrease in storage O_2 and increase in storage CO_2 concentrations are considered to promote GABA production at 25°C and our results indicate that the effect is influenced by storage temperature. Makino et al.[7] reported that an adjusted aerobic atmosphere is effective for stimulating GABA production in tomato fruits at 30°C. However, this temperature is rather high for storing fresh produce. Futhermore, Gautier et al.[8] reported that suppression of normal ripening was observed when temperatures increased from 27°C to 32°C in experiments using green mature tomato fruits. In the present study, we demonstrated that GABA production in tomato fruits can be promoted at 25°C. Among the atmospheres tested in the current study, GABA production in tomato fruits was found to be stimulated the most under hypoxic conditions created by the high barrier pouches (Fig.2); this is similar to the observations made in a study on tea[15]. However, this atmosphere is not suitable

for storing fresh produce, as mentioned above.

Glutamate concentrations were significantly higher in fruits stored in macro-perforated pouches than in the other pouches after 12.6-d storage at 15°C and after 3.9-d storage at 25°C (Fig.3). Notably, the concentration was significantly lower in the high barrier pouch than in the other pouches at 6.6-d storage at 25°C. If glutamate, a main substrate in the glutamic acid decarboxylase (GAD) reaction, is consumed to produce GABA, the glutamate concentration would decrease with GABA accumulation, as has been demonstrated in radish leaves[16].

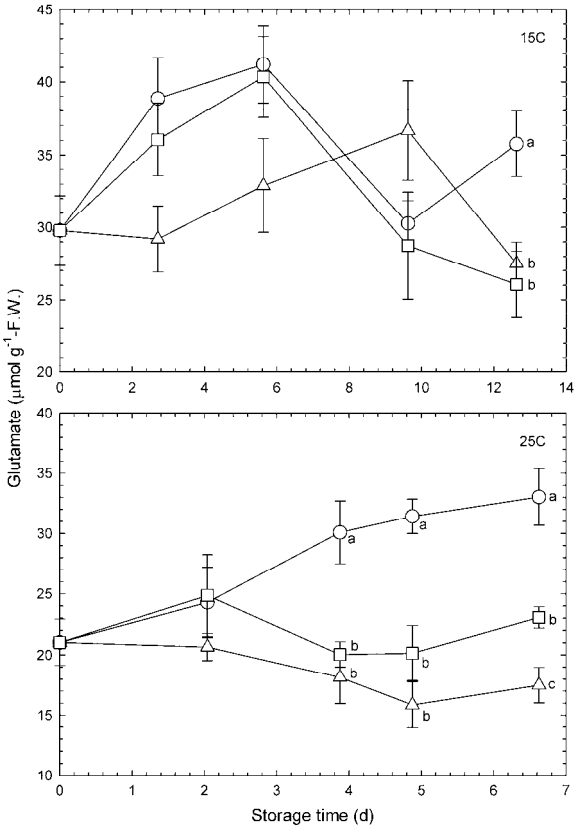

Fig.3 Changes in glutamate in tomato fruits at 15 and 25℃ (n = 6). Circles, triangles, and squares denote values under ambient air, hypoxic conditions with high CO_2 levels, and adjusted aerobic (low O_2 and high CO_2 conditions) atmospheres, respectively. Mean ± SE of six observations are plotted. Within the same time interval, symbols followed by the same letter are not significantly different ($P > 0.05$; one-way ANOVA with LSD test).

The glutamate concentrations after 6.6-d storage at 25°C were inversely proportional to the GABA concentrations. This reaction may be associated with the difference in GABA concentrations among fruits contained in the three kinds of pouches. However, an increase in glutamate molecules did not correspond with a reduction in GABA molecules. Glutamate is produced from α-ketoglutarate by glutamate and α-ketoglutarate-dependent GABA transaminase reactions, and is being consumed by the GAD reaction[17]. This relationship is not fully understood because of the complex metabolic pathways involved. Streeter and Thompson[16] suggested that glutamate may be generated by the decomposition of proteins in long-term incubation. Therefore, they incubated trimmed radish leaves for 6 h at room temperature and detected a decrease in glutamate with an increase in GABA. In the current study, intact tomato fruits were stored for 6.6 d at 25°C and for 12.6 d at 15°C. Liu and Luh[18] reported that concentrations of GABA and glutamate in tomato paste from harvest-delayed fruit were elevated due to delayed harvest. Differences in sample storage conditions appear to be the cause of differences in the glutamate data described above.

Alanine concentration was significantly lower

in the macro-perforated pouch than in the other pouches at 9.6-d storage at 15°C (Fig.4). This result reflects those of Streeter and Thompson[16] who reported that alanine and GABA accumulated in radish leaves under anoxic/hypoxic conditions. At 25°C, alanine concentration was significantly higher in the micro-perforated pouch than that in the other pouches after 3.9-d storage (Fig.4). Makino et al.[7] reported that alanine production is more stimulated under an adjusted aerobic atmosphere than that under hypoxic conditions at 30°C and this was shown at 25°C in the present study. Miyashita et al.[19] reported that O_2 stress promoted the decomposition of accumulated alanine-by-alanine aminotransferase (AlaAT) (EC 2.6.1.2) in Arabidopsis (*Arabidopsis thaliana* (L.) Heynh.). This suggests that alanine may decompose under hypoxic conditions through strong O_2 stress and may be maintained under adjusted aerobic atmospheres through weak O_2 stress. The accumulation of alanine in tomato fruits under the adjusted aerobic atmosphere supports the observation that atmospheric conditions stimulate GABA production[16]. At 15°C, the alanine concentration was not significantly different among three atmospheres, apart from at 9.6 d. This suggests that dynamic changes in alanine concentration are affected by the storage temperature as well as GABA concentration.

At 25°C, GABA production in vine-ripened tomato fruits under low O_2 and high CO_2 conditions (adjusted aerobic atmosphere) was significantly more stimulated than that in air. The

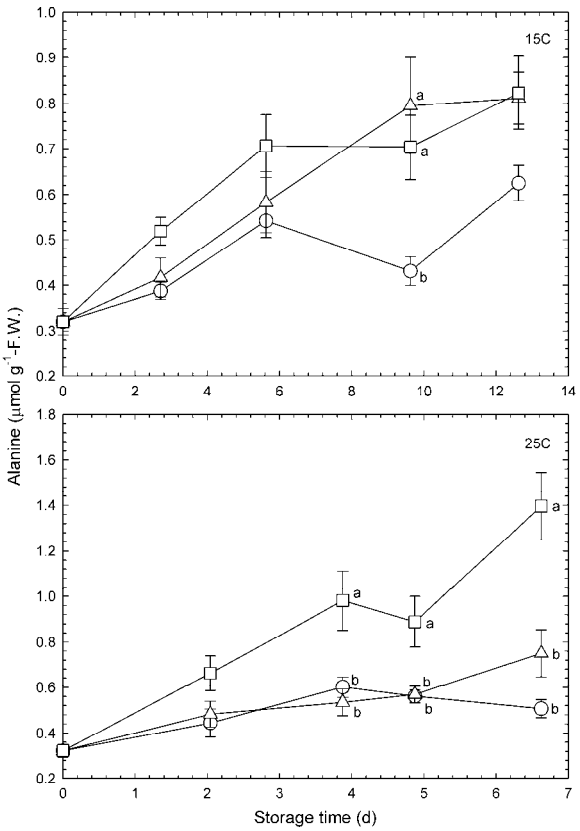

Fig.4 Changes in alanine in tomato fruits at 15 and 25℃ (n = 6). Circles, triangles, and squares denote values under ambient air, hypoxic conditions with high CO_2 levels, and adjusted aerobic (low O_2 and high CO_2 conditions) atmospheres, respectively. Mean ± SE of six observations are plotted. Within the same time interval, symbols followed by the same letter are not significantly different ($P > 0.05$; one-way ANOVA with LSD test).

accumulation of alanine under the adjusted aerobic atmosphere supports the observation that this atmosphere type is effective for stimulating GABA production. The atmosphere and temperature conditions may be applied to the storage and distribution of tomato fruits to

produce high GABA concentrations, thus enhancing the value of the fruit.

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