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Amino
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Cycling treatment of anaerobic and aerobic incubation increases the content of γ-aminobutyric acid in tea shoots

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Summary. γ-Aminobutyric acid (GABA), a hypotensive compound, is formed from glutamic acid under anaerobic condition in tea shoots. Glutamic acid was exhausted in the first three hours of anaerobic incubation and the increase of GABA stopped. After that, when tea shoots were released under aerobic condition, glutamic acid reproduced rapidly. After one hour of aerobic incubation, tea shoots were given three hours of anaerobic incubation again and then accumulated glutamic acid changed to GABA. The content of GABA increased much more than usual anaerobic incubation. GABA was more in the tea stem than in the leaf.

Keywords: Amino acids – γ-Aminobutyric acid (GABA) – Glutamic acid – Tea – Anaerobic incubation – Aerobic incubation – Stem

Introduction

The accumulation of γ-aminobutyric acid (GABA) under anaerobic condition is observed in many plants. Tea (*Camellia sinensis*) is not an exception (Tsushida and Murai, 1987; Tsushida et al., 1987). GABA is known to induce a fall in blood pressure. Omori et al. (1987) found the tea accumulated GABA induced a fall in blood pressure in spontaneously hypertensive rats. GABA is formed from glutamic acid by glutamate decarboxylase under anaerobic condition in tea shoots. We found the new fact that the amount of GABA was more increased by the repeating treatment of anaerobic and aerobic incubation than by only anaerobic incubation. And we investigated the amount of GABA in tea leaf and stem given the repeating treatment in this work.

Material and methods

Material

Fresh tea shoots of cv. Yabukita were plucked at the National Research Institute of Vegetables, Ornamental Plants and Tea, Kanaya, Shizuoka, Japan on April 27, 1999.

Leaf incubation

Plucked tea shoots were packed in nitrogen gas and incubated under anaerobic condition for three hours. Then tea shoots were exposed to the air and incubated under aerobic condition for one hour. These anaerobic incubation for three hours and aerobic incubation for one hour were cycled three times. After that the tea shoots were incubated under anaerobic condition for three hours lastly. This treatment lasted for 15 whole hours. Treated each samples were dried in microwave oven and then separated into leaves and stems. They are subjected to the analysis of individual free amino acids. The amounts of amino acids were compared to those of anaerobically incubated for 15 hours continuously.

Analysis of individual free amino acids

Dried tea samples were ground and extracted with hot water (Takayanagi et al., 1989) and tannin was removed by polyvinylpolypyrrolidone. Individual free amino acids were analyzed by high performance liquid chromatography (HPLC, Hitachi, L-6000). Alanine, GABA, arginine, aspartic acid, asparagine, glutamic acid, glutamine, serine and theanine (L-*N*-ethyl-glutamine) were analyzed by reversed phase HPLC with pre-column derivatization with *o*-phthalaldehyde at pH 9.0 and fluorescence detection using homoserine as an internal standard. To separate amino acids, an octadecylsilane (ODS) column (Develosil ODS-HG, 4.6 mm I. D. $\times$ 150 mm, Nomura Chem. Co., Seto) and a multi-step linear gradient of the concentration of acetonitrile in citrate buffer was used (Goto et al., 1993; Goto et al., 1996).

Results and discussion

First of all, fresh tea shoots were dried and separated into leaves and stems. Their individual free amino acids were analyzed (Table 1). Aspartic acid, glutamic acid, glutamine, arginine and theanine were major amino acids in tea shoot. Some amino acids were contained characteristically higher in tea stem than in the leaf. Theanine and glutamine in tea stem were about 3.4 and 11.6 times as much as in the leaf respectively.

The changes of the contents of aspartic acid, glutamic acid, alanine and GABA under anaerobic and aerobic conditions were shown in Fig. 1. During anaerobic incubation, the contents of glutamic acid and aspartic acid were decreased and those of GABA and alanine were increased. These changes were remarkable in the first three hours of anaerobic incubation. And in the

Table 1. Amino acids in tea leaf and stem (mg/g)

Amino acid	Leaf	Stem
Aspartic acid	1.65 $\pm$ 0.12	1.96 $\pm$ 0.13
Glutamic acid	2.46 $\pm$ 0.61	2.12 $\pm$ 0.12
Asparagine	0.08 $\pm$ 0.01	0.16 $\pm$ 0.03
Serine	0.71 $\pm$ 0.05	0.56 $\pm$ 0.02
Glutamine	0.60 $\pm$ 0.08	6.94 $\pm$ 1.46
Arginine	1.64 $\pm$ 0.04	1.54 $\pm$ 0.21
Alanine	0.12 $\pm$ 0.01	0.18 $\pm$ 0.03
Theanine	7.90 $\pm$ 0.47	27.21 $\pm$ 1.17
γ-Aminobutyric acid	0.03 $\pm$ 0.05	0.09 $\pm$ 0.03

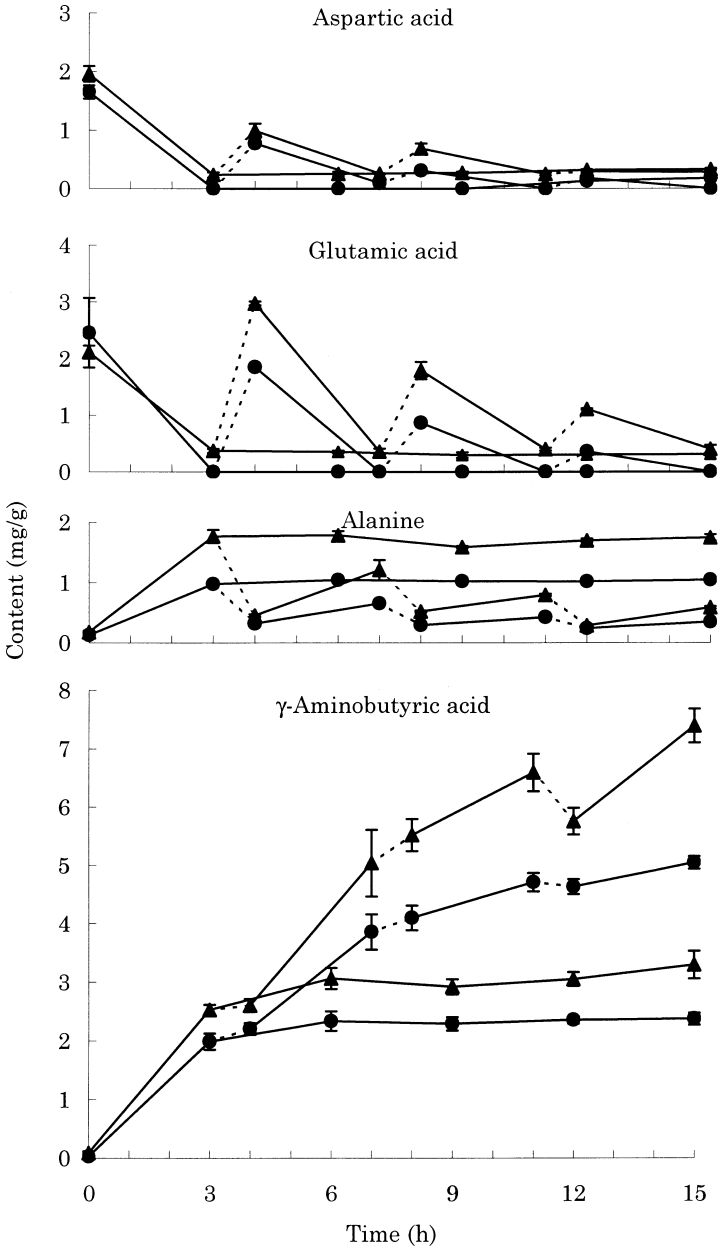

Fig. 1. Contents of amino acids in tea stem and leaf during anaerobic and aerobic incubation. ▲, stem; ●, leaf. —, anaerobic incubation; ···, aerobic incubation

following anaerobic incubation, the contents of those amino acids remained almost constant. The contents of GABA and alanine were higher in tea stem than in the leaf under anaerobic incubation.

Glutamic acid had been almost exhausted for the production of GABA in the first three hours of anaerobic incubation. But after that, when tea shoots were released under aerobic condition, glutamic acid was reproduced rapidly and recovered by its original amount only in one hour. On the other hand, the content of GABA didn't change much. Glutamic acid accumulated during

aerobic incubation changed to GABA in following three hours of anaerobic incubation. These aerobic incubation for one hour and anaerobic incubation for three hours were cycled three times. This repeating treatment for total 15 hours made accumulate GABA about two times as much as continuous anaerobic incubation for same 15 hours. The contents of GABA were higher in tea stem than in the leaf under the repeating treatment. The contents of reproduced glutamic acid were also higher in tea stem than in the leaf. Therefore, this method repeating anaerobic and aerobic incubation can accumulate GABA in tea shoot richer than previous continuous anaerobic incubation (Sawai et al., 1999). And the tea stem is important because it contain much GABA (Sawai et al., 1999).

While the content of GABA increased continuously during the repeating treatment of anaerobic and aerobic incubation, the content of alanine in tea shoot treated repeatedly did not exceed that treated only anaerobically. The content of alanine decreased remarkably during one hour of aerobic incubation. Aspartic acid was not also reproduced much under aerobic condition.

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References

Goto T, Horie H, Mukai T (1993) Analysis of major amino acids in green tea by high-performance liquid chromatography coupled with OPA precolumn derivatization. Tea Res J 77: 29–33 (Japanese)

Goto T, Yoshida Y, Amano I, Horie H (1996) Chemical composition of commercially available Japanese green tea. Foods Food Ingredients J Jpn 170: 46–52

Omori M, Yano T, Okamoto J, Tsushida T, Murai T, Higuchi M (1987) Effect of anaerobically treated tea (Gabaron tea) on blood pressure of spontaneously hypertensive rats. Nippon Nôgeikagaku Kaishi 61: 1449–1451 (Japanese)

Sawai Y, Konomi K, Odaka Y, Yoshitomi H, Yamaguchi Y, Miyama D, Takeuchi A (1999a) Repeating treatment of anaerobic and aerobic incubation increases the amount of γ-aminobutyric acid in tea shoots. Nippon Shokuhin Kagaku Kogaku Kaishi 46: 462–466 (Japanese)

Sawai Y, Konomi K, Odaka Y, Yoshitomi H, Yamaguchi Y, Miyama D (1999b) Contents of γ-aminobutyric acid in stem of anaerobic incubated tea shoot. Nippon Shokuhin Kagaku Kogaku Kaishi 46: 274–277 (Japanese)

Takayanagi H, Anan T, Ikegaya K (1989) Determination of amino acids in tea by high-performance liquid chromatography. Tea Res J 69: 29–34 (Japanese)

Tsushida T, Murai T (1987) Conversion of glutamic acid to γ-aminobutyric acid in tea leaves under anaerobic conditions. Agric Biol Chem 51: 2865–2871

Tsushida T, Murai T, Omori M, Okamoto J (1987) Production of a new type tea containing a high level of γ-aminobutyric acid. Nippon Nôgeikagaku Kaishi 61: 817–822 (Japanese)

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