Effects of anaerobic fermentation in a nitrogen atmosphere on bioactive compound content in Vietnamese GABA tea

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Received 6 May 2018; accepted 31 July 2018

Abstract:

Gamma(γ)-Aminobutyric acid (GABA), which provides a variety of health benefits, is an important bioactive component of tea (*Camellia sinensis*). A special tea product, GABA tea, contains high levels of γ-Aminobutyric acid which is produced by anaerobic fermentation. Tea fermentation is affected by a number of factors, including anaerobic conditions (vacuum, and the kind of gas used), temperature, and the duration of anaerobic fermentation.

In this study, the levels of bioactive compounds in tea leaves produced by fermenting fresh tea leaves in nitrogen gas are investigated. The fermentation was conducted at different temperatures: room temperature (28±2), 35, and 40°C; and for each temperature setting, the fermentation times were 6, 9, 12, and 15 hours. The results show that the GABA content increased significantly with fermentation temperature. The most appropriate temperature and time for the anaerobic fermentation to produce GABA and other bioactive compounds were, respectively, 40°C and 9 hours. Under these conditions, GABA content reached the maximum value (202.77 mg/100 g of dry tea); that of total polyphenols was 22.30% of dry matter (DM); caffeine was 2.39% DM; and soluble solids were 36.25% DM.

Keywords: **anaerobic fermentation, gamma-aminobutyric acid, nitrogen gas, tea.**

Classification number: **3.1**

Background

γ-Aminobutyric acid - GABA, $C_4H_9NO_2$, a non-proteinaceous amino acid, is one of three bioactive compounds in tea (the other two are caffeine and L-Theanine). GABA is one of the major inhibitory neurotransmitters in the sympathetic nervous system, exerting antihypertensive and antidiabetic effects in humans. GABA can act effectively as a natural relaxant to induce relaxation and diminish anxiety, and its administration can enhance immunity in stressful conditions [1]. Furthermore, GABA has a physiological role in many bodily systems outside the central somatic system, such as regulating cardiovascular functions, inhibiting metastasis of cancer cells, and modulating renal function. Kanehira, et al. [2] suggest that intake of GABA-containing beverages, especially those containing 50 mg of GABA, may help reduce both psychological and physical fatigue and improve task-solving ability.

Tea is a beverage that is widely appreciated and consumed in vast quantities worldwide. Tea products have been studied as functional foods for many years. A special tea product, GABA tea, which contains a large amount of GABA was discovered Japan in 1987 by Tsushida and Murai, and has been reported to have some medical functions [3-6]. The key difference between GABA tea and other tea products is the complicated anaerobic fermentation during withering operations [7]. The quality of a commercialised GABA tea is determined by the GABA content (at least 150 mg GABA/100 g dried tea) and its flavour [8]. Recently, GABA tea has been successfully produced in Taiwan. Tea farmers

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in Taiwan combine the technique of making Olong tea and green tea to produce the Taiwanese GABA tea which has a uniquely characteristic taste and flavour. GABA tea is becoming increasingly popular in Taiwan.

The information necessary for producing GABA tea in Vietnam remains unknown [5]. GABA content increases with the use of anaerobic fermentation. Recently, research on anaerobic fermentation in a vacuum has been published [9]. The fermentation in the current study was undertaken in nitrogen atmosphere to ensure a completely anaerobic medium [4, 10]. This study aims to improve the flavour and GABA content of GABA tea. The results will be provided to tea producers to improve GABA tea production technology. The purpose of this study is to determine the levels of certain bioactive compounds - caffeine, soluble solids, polyphenols, and GABA - in tea leaves under conditions of continuous anaerobic fermentation in a nitrogen atmosphere.

Materials and methods

Materials

Tea shoots consisting of a bud and 2-3 leaves of the LDP2 cultivar (*C. sinensis* x *C. sinensis* var. *assamica*) were used as raw material. These were collected from the Anh Son district (Nghe An province, Vietnam) in April 2017. Folin-Ciocalteau's phenol reagent, GABA (Sigma-Aldrich, USA), gallic acid (Sigma-Aldrich, USA), and other analytical-grade chemicals were used for biochemical compound analysis.

Methods

Experimental method:

After picking, tea shoots were spread on bamboo tray with a diameter of 1.1 m and placed in a cool room to wither for 4 hours before fermentation. Next, half of the plucked tea shoots were packed into a nitrogen-filled chamber and then incubated continuously at room temperature (28±2ºC), at 35ºC, and at 40ºC. For each temperature, fermentation times of 6 hours, 9 hours, 12 hours, and 15 hours were applied. The other half of the tea shoots were not subjected to anaerobic treatment and were used as the control.

The GABA tea production process can be summarily described as follows: fresh tea shoots → withering (25-30ºC) → anaerobic fermentation → panning (5 minutes) → rolling (45 minutes) → drying (95ºC for 60 minutes) → final products.

The samples were collected at the end of each fermentation period and were kept in hermetic packaging and stored in dry, cool place before analysis. The soluble solid, polyphenol, caffeine, and GABA content were used as observation parameters.

Methods of analysis of biochemical components:

Soluble solid content was determined by Voronsov's method. Caffeine content was determined using the method of Bertrand [11]. Determination of total polyphenol content was conducted by means of a colorimetric method using Folin-Ciocalteau's reagent [12]; and GABA content was also determined by a colorimetric method [13, 14].

Statistical analysis:

Statistical analysis was performed using SAS software 9.1. A significance level of 0.05 was applied.

Results and discussion

Effect of anaerobic fermentation time and temperature on soluble solid content

The results in Table 1 show that the content of dissolved substances decreases when anaerobic fermentation time increases. However, after 15 hours of fermentation, the concentration of solutes seemed to be stable; the value for

Table 1. Soluble solid content (% DM) in tea obtained by anaerobic fermentation in a nitrogen gas environment.

Temperature	Anaerobic fermentation time				
	Control	*6 hours*	*9 hours*	*12 hours*	*15 hours*
28°C	40.27^{a}	36.52^{b}	35.11^{bc}	33.62^{c}	31.70^{d}
35°C	40.27^{a}	38.39^{b}	37.58^{b}	35.20^{c}	33.29^{d}
40°C	40.27^{a}	37.60^{b}	36.25^{c}	35.46^{c}	31.98^{d}

Values with different superscripts in a row are significantly different ($p<0.05$).

fermentation at 28°C was 31.70% DM. The same trend was observed for temperatures of 35°C and 40°C. Soluble dry-matter reduction in tea may be due to the anaerobic metabolism of micro-organisms during fermentation, and degradation through enzymatic and non-enzymatic reactions that contribute to the taste and colour of the tea blocks [15-18]. It can be deduced that a short anaerobic period leads to less soluble matter loss. Thus, the duration of anaerobic fermentation needs to be limited to minimise soluble matter loss.

Effect of anaerobic fermentation time and temperature on total polyphenol content

Green tea contains polyphenol compounds, which include flavanols, flavandiols, flavonoids, and phenolic acids. Most of the polyphenols in green tea are flavanols, commonly known as catechins. Table 2 shows the total polyphenol content of tea leaves under different anaerobic conditions. The total polyphenol content of fermented tea leaves was significantly lower than that control sample ($p<0.05$). After 6 hours of anaerobic fermentation at room temperature, polyphenol content decreased significantly compared to that of the untreated sample ($p<0.05$). This trend was similar to that of the anaerobic fermentation conducted at 35°C and 40°C. Specifically, the total polyphenol content decreased from 22.30% to 20.42% DM after 9 hours of anaerobic fermentation at 40°C and continued to decline significantly after 12 hours and 15 hours of fermentation. This suggests that the polyphenols were used for the biosynthesis of other chemical components during the anaerobic fermentation. Thus, it is necessary to control fermentation time in order to minimise the loss of these antioxidants which are beneficial to human health.

Effect of anaerobic fermentation time and temperature on caffeine content

Caffeine is also found in tea leaves. The proportion varies from 2.5% to 5.5% in dry leaves, depending on their origin, age, and processing. Caffeine continues to be one of the most popular products used as an energy stimulant and as an antioxidant. Table 3 shows the caffeine content of the tea leaves when different anaerobic treatment temperatures and times are used. For all fermentation temperatures, caffeine content decreased slightly after 6 hours ($p>0.05$) and then significantly after 12 hours ($p<0.05$). The caffeine content of the leaves depends on the fermentation conditions. In a carbon dioxide atmosphere, caffeine content decreases; in a nitrogen atmosphere, it increases; and it seems unchanged in an oxygen atmosphere [4]. Microorganisms can cause caffeine degradation through demethylation. The major metabolite formed in fungi is theophylline (1,3-dimethylxanthine), whereas theobromine (3,7-dimethylxanthine) is the major metabolite in bacteria [17, 18].

Table 2. Total polyphenol content (% DM) in tea obtained by anaerobic fermentation in a nitrogen gas environment.

Temperature	Anaerobic fermentation time				
	Control	*6 hours*	*9 hours*	*12 hours*	*15 hours*
28°C	24.66^{a}	23.27^{b}	22.58^{b}	21.32^{c}	20.03^{d}
35°C	24.66^{a}	23.10^{b}	22.88^{bc}	21.65^{c}	19.87^{d}
40°C	24.66^{a}	23.46^{b}	22.30^{c}	20.42^{d}	19.58^{d}

Values with different superscripts in a row are significantly different ($p<0.05$).

Table 3. Caffeine content (% DM) in tea obtained by anaerobic fermentation in a nitrogen gas environment.

Temperature	Anaerobic fermentation time				
	Control	*6 hours*	*9 hours*	*12 hours*	*15 hours*
28°C	2.74^{a}	2.28^{b}	2.15^{b}	2.03^{c}	1.84^{d}
35°C	2.74^{a}	2.34^{b}	2.18^{b}	2.13^{b}	1.92^{c}
40°C	2.74^{a}	2.50^{b}	2.39^{bc}	2.23^{c}	2.05^{d}

Values with different superscripts in a row are significantly different ($p<0.05$).

Table 4. GABA content (mg/100 g dry tea) in tea obtained by anaerobic fermentation.

Temperature	Anaerobic fermentation time				
	Control	*6 hours*	*9 hours*	*12 hours*	*15 hours*
28°C	66.07[c]	137.74[b]	167.04[a]	169.03[a]	170.95[a]
35°C	66.07[d]	139.35[c]	182.61[a]	183.95[a]	178.03[b]
40°C	66.07[d]	142.2[c]	202.77[ab]	211.31[a]	200.97[b]

Values with different superscripts in a row are significantly different ($p<0.05$).

Effect of anaerobic fermentation time and temperature on GABA content

GABA tea is one kind of functional tea manufactured by special methods in anaerobic conditions. It is referred to as GABA tea because it is rich in GABA, the acronym for the special amino acid it contains. The results show wide variation in most of the GABA content (Table 4). As shown in Table 4, the GABA content range depends on the temperature and fermentation time. The present study accords with those of Park [19] and Tsushida [4]. The GABA content was highest at a fermentation temperature of 40°C and a fermentation time of 9 to 15 hours (200.97-211.31 mg/100 g dry tea). It is at this temperature that the GAD enzyme works best [20]. However, after 12 hours of fermentation, the content of biologically active ingredients such as caffeine and polyphenols decreased significantly. Therefore, an anaerobic fermentation time of 9 hours at 40°C is the most suitable as it ensures the greatest quantities of GABA and other biologically active ingredients [6-8]. The highest GABA content obtained was 202.77 mg/100 g, from the treatment of 9 hours at 40°C [7, 9, 10]. The levels of biologically active compounds in GABA tea are ensured by Vietnamese national standards for commercial tea products (TCVN 1454-1993).

Conclusions

Results showed that GABA content in GABA tea had increased significantly after anaerobic fermentation in a nitrogen gas environment, reaching the standard of GABA tea. Changes in the quantities of tea polyphenols, caffeine, and soluble matter tended to gradually decrease as the fermentation time was extended. The most appropriate temperature and time for anaerobic fermentation in nitrogen gas were, respectively, 40ºC and 9 hours. Under these conditions, the GABA tea analysed possessed a high GABA content (202.77 mg/100 g of dry tea), and the quantities of other components met commercial product requirements (total polyphenol content was 22.30% DM; caffeine was 2.39% DM; and soluble solids were 36.25% DM). The presence of these active components is beneficial to the quality of the tea.

REFERENCES

[1] A.M. Abdou, S. Higashiguchi, K. Horie, M. Kim, H. Hatta, H. Yokogoshi (2006), "Relaxation and immunity enhancement effects of gamma-aminobutyric acid administration in humans", *Biofactors*, **26**, pp.201-208.

[2] T. Kanehira, Y. Nakamura, K. Nakamura, et al. (2011), "Relieving occupational fatigue by consumption of a beverage containing γ-aminobutyric acid", *J. Nutr. Sci. Vitaminol.*, **57**, pp. 9-15.

[3] Y. Abe, S. Umemura, K. Sugimoto, N. Hirawa, Y. Kato, N. Yokoyama (1995), "Effect of green tea rich in gamma-aminobutyric acid on blood pressure of Dah1salt-sensitive rats", *American Journal of Hypertension*, **8**, pp.74-79.

[4] T. Tsushida, T. Murai, M. Omori, J. Okamoto (1987), "Production of a new type tea containing a high level of γ-aminobutyric acid", *Nippon Nogeikagaku Kaishi*, **6**, pp.817-822.

[5] H.F. Wang, Y.S. Tsai, M.L. Lin, A.S.M. Ou (2006), "Comparison of bioactive components in GABA tea and green tea produced in Taiwan", *Food Chemistry*, **96**, pp.648-653.

[6] Zhen Zeng, Chunlan Wu, Yahui Huang, W. Juan (2012), "Study on flavour volatiles of γ-aminobutyric acid (GABA) green tea", *African Journal of Biotechnology*, **11(51)**, pp.11333-11341.

[7] Y. Sawai, Y. Yamaguchi, D. Miyama, H. Yoshitomi (2001), "Cycling treatment of anaerobic and aerobic incubation increases the content of γ-aminobutyric acid in tea shoots", *Amino Acids*, **20**, pp.331-334.

[8] A.S.M. Ou, S.F. Wang, C.Y. Wu, Y.S. Tsai (2005), "Investigation of optimum manufacturing condition and biological functions", *NSC project report*.

[9] Nguyen Quoc Sinh, Nguyen Van Toan, Do Thi Bich Thuy, Le Thanh Long, Ho Sy Vuong, Nguyen Thi Diem Huong, Nguyen

Cao Cuong, Vo Van Quoc Bao (2016), "Effects of anaerobic fermentation in vacuum conditions on bioactive compound content of GABA tea", *Journal of Agricultural Sciences and Technology*, Nong Lam University Ho Chi Minh city, **6**, pp.50-54.

[10] Mingyuan Lee, Jinchyau Peng (2010), "Effects of fermentation time and seasons on the γ-aminobutyric acid and glutamic acid contents of TTES-12 GABA tea producing", *International Symposium on Machinery and Mechatronics for Agriculture and Biosystems Engineering, Fukuoka, Japan*, pp.614-620.

[11] V.T. Thu, D.H. Tien, D.T. Gam, G.T. Khoa (2001), *Chemical compounds in tea and some popular analysis methods in tea production in Vietnam*, Hanoi Agricultural Publisher.

[12] The Ministry of Science and Technology of Vietnam (2005), *National standards for determining the quality characteristics of green tea and black tea - Part 1: total polyphenol content in tea - Colorimetric method using Folin's reagent-ciocalteu (ISO 14502-1:2005)*.

[13] Truong Nhat Trung, Dong Thi Anh Dao (2016), "Study of boosting gamma-Aminobutyric acid (GABA) content in germinated mung bean under hypoxia-anaerobic condition and evaluating the loss of GABA after boiling", *Science & Technology Development Journal (University of Technology, VNU-HCM)*, **7(19)**, pp.1-9.

[14] Qian Zhang, Jun Xiang, Lizhen Zhang, Xiaofeng Zhu, Jochem Evers, Wopke van der Werf, Liusheng Duan (2014), "Optimizing soaking and germination conditions to improve gamma-aminobutyric acid content in japonica and indica germinated brown rice", *Journal of Functional Foods*, **10**, pp.283-291.

[15] Katsuhiro Hakamata, Norio Nakada, Toshihiro Mukai, Hirokazu Fukushima, Ryo Yamaguchi, Tomofumi Nakada (1988), "Improvements of manufacturing process of anaerobically treated tea (Gabaron tea)", *Chagyo Kenkyu Hokoku*, **68,** pp.8-13.

[16] J.H. Kim, M.Y. Kim (2012), "Enhancement of bioactive components content and the antioxidant activity of green tea after continuous anaerobic incubation", *J. Agr. Sci. Tech.*, **14**, pp.837-844.

[17] S. Dash, S. Gummadi (2006), "Catabolic pathways and biotechnological applications of microbial caffeine degradation", *Biotechnol. Lett.*, **28,** pp.1993-2002.

[18] S. Ibrahim, M.Y. Shukor, M.A. Syed, N.A. Ab Rahman, K.A. Khalil. A. Khalid, S.A. Ahmad (2014), "Bacterial degradation of caffeine: a review", *Asian J. Plant Biol.*, **1**, pp.18-27.

[19] Jang Hyun Park (2001), "Change in the main constituents by a treatment condition of anaerobically treated green tea leaves", *Korean J. Medicinal Crop Sci*., **9**, pp.275-279.

[20] Nguyen Thi Hong Hanh, Trieu Ngoc Han, Le Nguyen Doan Duy and Nguyen Cong Ha (2016), "Changes of glutamic acid content and glutamate decacboxylase during the soaking and germination of pre-germinated brown rice", *Scientific Magazine of Can Tho University (Agriculture)*, **1**, pp.66-74.