LWT - Food Science and Technology 46 (2012) 64–70

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Bioactive components and antioxidant properties of γ-aminobutyric acid (GABA) tea leaves

Sheng-Dun Lin [a,*], Jeng-Leun Mau [b], Ching-An Hsu [a]

[a] Department of Food Science and Technology, Hungkuang University, 34 Chungchie Road, Shalu, Taichung 43302, Taiwan, ROC
[b] Department of Food Science and Biotechnology, National Chung-Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan, ROC

ARTICLE INFO

Article history:
Received 24 March 2011
Received in revised form
25 October 2011
Accepted 31 October 2011

Keywords:
GABA tea
Catechin
Theanine
γ-Aminobutyric acid
Antioxidant property

ABSTRACT

Various ethanolic concentrations (0–95%, v/v) and temperatures (25–95 °C) were used to extract γ-aminobutyric acid (GABA) tea leaves. Extraction yields, and contents of total phenols, various catechins, GABA, theanine, and antioxidant properties of extracts were determined. The 50% (v/v) ethanol at 50–95 °C gave higher yields (32.05–32.56 g dried extract/100 g dried tea leaves). The bioactive components and antioxidant properties of extracts were affected by the ethanolic concentrations and temperatures. Among catechins, epigallocatechin gallate was the main catechin in all extracts, followed by epigallocatechin, epicatechin, epicatechin gallate, gallocatechin and gallocatechin gallate. The 50–75% (v/v) ethanol at 75–95 °C gave higher contents of ester type (102.92–104.54 mg/g extract) and non-ester type (61.75–63.55 mg/g extract) catechins. Water at 50–75 °C gave higher GABA and theanine contents and higher chelating ability of extracts. The 75% (v/v) ethanol at 25–75 °C gave higher scavenging ability and reducing power of extracts. Based on dried tea extracts or leaves results obtained, the optimal extraction conditions to maintain the total contents of various catechins, GABA and theanine in the maximum level were 50% ethanol (v/v) and 75–95 °C.

1. Introduction

Tea is one of the most popular and widely consumed beverages in the world, and its consumption amount is comparable to that of coffee. In general, tea can be classified according to the aerobic incubation as unfermented-green tea, partially fermented-oolong tea, fully fermented-black tea and post-fermented-pu-erh tea. Black tea is commonly consumed in the Western world whereas the consumption of green tea, oolong tea and pu-erh tea are favoured in Asian countries, mainly for their attractive colour, pleasant aroma, good taste, and potential health benefits.

The γ-aminobutyric acid (GABA) tea is popular by consumers in Taiwan in recent years. The GABA tea, also called Gabaron tea, is the special tea enriched with GABA by anaerobic conditions of fresh tea leaves (Tsushida, Murai, Omori, & Okamoto, 1987). The amount of GABA was accumulated by the repeating treatment of anaerobic and aerobic incubation than by only anaerobic incubation, and GABA was higher in the tea stem than in the leaf (Sawai, Yamaguchi, Miyama, & Yoshitomi, 2001). The GABA is a non-proteinaceous amino acid and is one of the major inhibitory neurotransmitters in the central nervous system. The GABA could work effectively as a natural relaxant to induce relaxation and diminish anxiety, and its administration could enhance immunity under stress conditions (Abdou et al., 2006). Furthermore, GABA has a physiological role in many systems outside the central system, such as regulation cardiovascular functions, inhibition metastasis of cancer cells, and modulation renal function. The main difference between GABA and green tea is the contents of GABA, glutamic acid, alanine, aspartic acid, total catechins, epigallocatechin gallate (EGCG) and epicatechin (EC), especially the first two components (Wang, Tsai, Lin, & Ou, 2006). However, no difference is found in other components between two kinds of tea, and GABA tea is comparable to green tea in biological activity. The GABA-containing drinks made from GABA tea have recently become a popular drink among health-conscious individuals in Asian countries, and may also provide its beneficial effects.

In addition to GABA, several studies have shown that tea contains a number of bioactive components, especially polyphenolic compounds and theanine. The polyphenolic compounds, especially catechins, have many bioactive actions such as antioxidant, antimutagenic, anticarcinogenic, and antimicrobial activities (Almajano, Carbó, López Jiménez, & Gordon, 2008; Gupta, Siddique,

* Corresponding author. Tel.: +886 4 2631 8652x5038; fax: +886 4 2631 9176.
E-mail address: lin54@sunrise.hk.edu.tw (S.-D. Lin).

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doi:10.1016/j.lwt.2011.10.025

Beg, Ara, & Afzal, 2008; Lin, Liu, & Mau, 2008). Theanine (glutamic acid γ-ethyl amide; 5-N-ethyl glutamine) is a free form of non-proteinaceous amino acid found in tea and is very important because of its biological effects and flavour characteristics. For example, the amino acid was shown to increase serotonin, dopamine, and GABA levels in the brain and resulted in enhancing the neuroprotective effects (Kakuda, 2002; Nathan, Lu, Gray, & Oliver, 2006). Moreover, theanine lowered blood pressure in spontaneously hypertensive rats (Yokogoshi et al., 1995).

Type of solvent is an important parameter affecting the recovery of phytochemicals during extractions. Several solvents, such as ethanol, methanol, acetone, propanol and ethyl acetate, have been used for the extraction of polyphenols from plant materials (Alothman, Bhat, & Karim, 2009). Active ingredients in tea leaves, mainly catechins, are usually extracted with organic solvents and the conditions for extraction greatly affected the yield and quality of extracts obtained (Perva-Uzunalić et al., 2006). However, ethanol is non-toxic, easy to be recycled and mixed with water in different ratios and the application of ethanol and water for human consumption is acceptable. Besides, the extraction solvent should be low-cost and safe for both the operating personnel and the consumers from the industrial-scale production viewpoint. Thus, aqueous ethanol is used to extract phenols from plant materials (Alothman et al., 2009; Durling et al., 2007; Xi et al., 2009). Extraction temperature is another important parameter needed to be optimised during extraction (Kim, Murthy, Hahn, Lee, & Paek, 2007). An increase in the working temperature generally favours both the solubility of solute and the diffusion coefficient, but beyond a certain value phenolic compounds can be denatured (Spigno & De Faveri, 2007).

There is little information available regarding the optimal extraction conditions of GABA tea on bioactive components and antioxidant properties. Therefore, the purpose of this study was to determine the effects of various concentrations of ethanol and extraction temperatures on the extraction yields, total phenols, various catechins, GABA and theanine of GABA tea leaves. Antioxidant properties of extracts from different extraction conditions were also determined.

2. Materials and methods

2.1. Materials

The GABA tea was made from the leaves of *Camellia sinensis* L. (cultivar Shy-Jih-Chun) in the winter season and leaves were picked from the tea farm in Mingjian, Nantou County, Taiwan. For GABA tea, young leaves were put into a nitrogen-filled chamber for 8 h and then shaken continuously under aerobic conditions for 3 h. The two steps were repeated twice, followed by a further anaerobic fermentation (8 h), prior to blanching (250–270 °C, 6 min), rolling and drying. The GABA tea was ground in a mill (RT-30HS, Rong Tsong Precision Technology Co., Taichung, Taiwan), and screened through a 0.5 mm sieve.

Methanol, acetonitrile and phosphoric acid were purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). Catechin, EC, gallocatechin (GC), epigallocatechin (EGC), catechin gallate (CG), epicatechin gallate (ECG), gallocatechin gallate (GCG), EGCG, GABA, theanine, *o*-phthaldialdehyde, 2-mercaptoethanol, Folin-Ciocalteu's phenol reagent, trifluoroacetic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), trichloroacetic acid, potassium ferricyanide, ferrous chloride, ferrozine, ascorbic acid, α-tocopherol, butylated hydroxyanisole (BHA), ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Anhydrous sodium carbonate was purchased from Shimakyu's Pure Chemicals (Osaka, Japan). Sodium phosphate and ferric chloride were purchased from Wako Pure Chemical Industries Co. (Osaka, Japan). Sodium acetate was purchased from Katayama Chemical Industries Co. (Osaka, Japan). Ethanol was purchased from Taiwan Tobacco & Liquor Co. (Tainan, Taiwan). Hydrochloric acid was purchased from Union Chemical Works Ltd. (Hsinchu, Taiwan).

2.2. GABA tea extracts preparation

The aqueous and ethanolic extracts from GABA tea leaves were prepared by extracting ground tea powder (10 g) with 100 mL of aqueous or various concentrations (25, 50, 75 and 95%, v/v) of ethanol in a shaking bath (25, 50, 75 and 95 °C) (SB302, Kansin Instruments Co., Kaohsiung, Taiwan) at 100 rpm for 30 min, and then centrifuged at 6000 rpm for 10 min (3460 g), filtered through Advantec No.1 filter paper (Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The residue was re-extracted with additional 100 mL portions of solvent as described above. The combined filtrates were rotary evaporated at 40 °C and then freeze-dried. Dry extracts thus obtained were stored at −20 °C before use. The aqueous, 25, 50, 75 and 95% (v/v) ethanolic extracts were prepared from GABA tea leaves at 25 °C were designated as 25C0E, 25C25E, 25C50E, 25C75E and 25C95E, respectively. The aqueous and various ethanolic extracts were prepared from GABA tea leaves at 50 °C were in turn designated as 50C0E, 50C25E, 50C50E, 50C75E and 50C95E, respectively. The aqueous and various ethanolic extracts were prepared from GABA tea leaves at 75 °C were in turn designated as 75C0E, 75C25E, 75C50E, 75C75E and 75C95E, respectively. The aqueous and various ethanolic extracts were prepared from GABA tea leaves at 95 °C were in turn designated as 95C0E, 95C25E, 95C50E and 95C75E, respectively. All treatments were randomly produced and conducted in triplicate.

2.3. Determination of total phenols

Total phenols were determined according to the method of Julkunen-Tiitto (1985) with minor modification. Each extract (100 mg) was dissolved in 75% (v/v) ethanol (5 mL) using an ultrasonic bath (T 710DH, Elma Hans Schmidbauer GmbH & Co. KG, Germany) with 40 kHz for 3 min, and then the volume was adjusted to 10 mL. The resulting mixture (100 μL) was diluted with water (2 mL) in a 10 mL volumetric flask. One millimeter of Folin-Ciocalteu's phenol reagent was added and the flask was vigorously shaken. Immediately, 5 mL of 20 g/100 mL sodium carbonate solution was pipetted and the mixture made up to 10 mL, shaking thoroughly again. After 20 min of standing, the mixture was centrifuged at 3000 rpm for 10 min (1400 g), the absorbance of the clear mixture was measured at 735 nm against a blank. The content of total phenols was calculated on the basis of the calibration curve of gallic acid.

2.4. Determination of various catechins

Various catechin contents in extracts were determined according to the method of Liang, Ma, Lu, and Wu (2001) with minor modification. Each extract (100 mg) was dissolved in 75% (v/v) ethanol (5 mL) using an ultrasonic bath with 40 kHz for 3 min, and then the volume was adjusted to 10 mL. The solution was filtered using a PVDF syringe filter (13 mm × 0.45 μm) before injection into an HPLC system consisting of a Hitachi L-2130 pump, a Hitachi L-2400 UV detector, and a Purospher® STAR RP-18e column (250 mm × 4 mm × 5 μm, Merck Co., Germany). Mobile phase solvent A consisted of acetonitrile/acetic acid/water (6:1:193, v/v/v). Mobile phase solvent B consisted of acetonitrile/acetic acid/water (60:1:193, v/v/v). The gradient program is eluting 100%