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A fermented tea with high levels of gallic acid processed by anaerobic solid-state fermentation

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ABSTRACT

Microbial tea products are widely welcomed for their unique quality characteristics. However, the quality characteristics of pickled tea, a microbial tea product traditionally consumed in most Asian countries remain unclear. Therefore, the quality characteristics of pickled tea processed using *Camellia sinensis* leaves through anaerobic solid-state fermentation were analyzed. During 60-d fermentation, the free amino acids, caffeine, and tea polyphenols in pickled tea kept much stable, the soluble sugar content showed significant and continuous decrease, but the water extract showed a continuously increasing trend. The optimal fermentation time for pickled tea was 30 d, at which its normal quality characteristics were formed: the sensory quality was the best, most free amino acids evidently increased, a high level of gallic acid was produced up to 25.7 g/kg, and volatile compounds were mainly alkanes such as heptadecane and hexadecane. It is the first report that high levels of gallic acid in a microbe-fermented tea processed by anaerobic fermentation reached to that levels in *Galla chinensis*, which will benefit development of a functional tea with high levels of active components and understanding the microbial transformation of tea components through anaerobic fermentation.

1. Introduction

Tea is one of the most popular natural beverages worldwide, and there are many health benefits of drinking tea such as enhancing immunity, quenching thirst, and reducing cholesterol (Yang & Lambert, 2011). Among tea products, dark teas such as Pu-erh tea, and Fuzhuan brick tea constituted to most of microbe-fermented tea, are mainly produced by aerobic fermentation. However, pickled tea, one of microbe-fermented teas, is obviously different from most of dark teas in that its fermentation is under anaerobic conditions (Nanba et al., 1998). Pickled tea has different names in different countries, such as Yancha or Suancha in China (Nanba et al., 1998), Lahpet tea, Laphet, or Leppet tea-so in Myanmar (Nanba, Nyein, Win, & Miyagawa, 1999), Miang tea in Thailand and Laos (Reichart, Philipsen, Mohr, Geerlings, & Srisuwan, 1988), and Goishi-cha, and Awaban-cha in Japan (Kato et al., 1994). Pickled tea mostly is eaten directly as a snack, a side dish or a salad in China, Thailand, Laos, Myanmar and Singapore (Nanba et al., 1999), but its dried form is brewed completely in Japan (Kato et al., 1994).

There were some reports on pickled tea; however, most of these studies focused on the isolation and identification of associated microbial strains, particularly lactic acid bacteria (Kato et al., 1994; Klayraung & Okonogi, 2009; Klayraung, Viernstein, Sirithunyalug, & Okonogi, 2008; Okada, Daengsubha, Uchimura, Ohara, & Ki, 1986; Sumalee, Nathachai, & Meechui, 2010; Tanasupawat, Pakdeeto, Thawai, Yukphan, & Okada, 2007; Xiao, Huang, Yang, Zhang, & Quan, 2015). Besides the isolation of the strains, the antibacterial and antioxidant activities, and the properties relevant to probiotic action of lactobacilli isolated from Miang tea have also been investigated (Tanasupawat et al., 2007; Klayraung & Okonogi, 2009; Klayraung et al., 2008). Recently, the catechin composition of Miang tea was analyzed (Sumalee et al., 2010). The components and strains of Goishi-cha were also reported (Kato et al., 1994), and resorcinol isolated from Awaban-cha was identified as a novel tea-based antioxidant (Hiasa et al., 2013). However, little research has

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been conducted on the quality of pickled tea, and its quality characteristics are still not much clear (Kato et al., 1994; Klayraung & Okonogi, 2009; Klayraung et al., 2008; Nanba et al., 1998; Nanba et al., 1999; Okada et al., 1986; Reichart et al., 1988; Sumalee et al., 2010; Tanasupawat et al., 2007).

At present, microbe-fermented tea is preferred by many people and becoming increasingly popular in that their unique quality characteristics are formed through microbial fermentation (Lv, Zhang, Lin, & Liang, 2013; Lv et al., 2012; Mo, Zhu, & Chen, 2008; Zhang, Zhang, Zhou, Ling, & Wan, 2013; Zhu et al., 2015). Furthermore, pickled tea provides refreshing and thirst-quenching effects, and is also beneficial to the human digestive system by regulating bile and mucus, which attract people to continually keep the habit of consuming pickled tea (Hirota, Ngatu, Miyamura, Nakamura, & Suganuma, 2011; Klayraung & Okonogi, 2009; Klayraung et al., 2008). We already identified *Lactobacillus plantarum* as the dominant strain during pickled tea fermentation (Xiao et al., 2015). Therefore, in order to clarify the quality characteristics of pickled tea, the quality characteristics of a pickled tea fermented by anaerobic solid-state fermentation were investigated, which will promote the development of a fermented tea with high levels of functional components.

2. Materials and methods

2.1. Tea leaves and reagents

Buds with three or four leaves of *Camellia sinensis* cv. Fudingdabai were plucked during summer and autumn at the tea plantation of Huazhong Agricultural University, Wuhan City, Hubei Province, China. All the reagents, such as ferrous sulfate, potassium sodium tartrate, disodium hydrogen phosphate, and stannous chloride, were purchased from Tianyuan Reagent Co., Ltd., Wuhan City, China and were of analytical grade.

2.2. Preparation of tea samples

All the fresh tea leaves were deactivated about 90 s in the boiling water, and were cooled by ice water immediately. The cooled leaves were fetched out to a clean bamboo plaque for water volatilisation by a fan. When the weight of the blanched leaves decreased about 40% compared with that of fresh leaves after 40–60 min, 5% (w/w) edible salt was added in proportion to the total leaf weight. The leaves mixed with edible salt were divided into several copies evenly, and each of them about 55–60 g in a 100-mL glass bottle was pressed very tightly and fully by hammering. The bottles were completely sealed, then divided into eleven groups randomly and placed at room temperature (25 ± 2 °C) in anaerobic solid-state fermentation. Each experiment was composed by eleven groups, and each group as a treatment was performed in a parallel experiment using three repeated bottles. The total number of the bottles in an experiment was 33. Each bottle was marked serial numbers, but they were placed randomly under the same conditions. The three replicates were performed in different period according to the same method.

Tea samples were collected from the marked bottles in 0, 6, 12, 18, 24, 30, 36, 42, 48, 54 and 60 d, respectively. Ten grams of each sample was used to measure pH value, and the remains were used to evaluate the sensory quality and then dry at 80 °C for 4 h for the component analysis. The samples fermented for 30 d with the best sensory qualities, were furthermore chosen for analysis of the quality characteristics such as free amino acid (FAA, initials of the first letter of each word) composition, catechin composition, gallic acid, tea pigments, volatile components. The tea samples collected in the 0 day were thought to have not been fermented by microbes, so they were also used as controls in this study.

2.3. Evaluation of the sensory qualities of pickled tea

In this study, to ensure an accurate assessment of the sensory quality of pickled tea, scoring criteria of color, aroma, flavor and texture characteristics were set up in advance (Suppl. 1). In the criteria, the scores were distributed according to a percentage, and the deduct marks were also listed. Tea sample was placed in a 400-cm^2 white square plate to evaluate the sensory qualities of pickled tea. The procedure for sensory evaluation is as follows: tea sample → smelling aroma → evaluating color and posture → evaluating texture and flavour by chewing pickled tea. The sensory score was marked according to the scoring criteria used to evaluate the sensory quality of pickled tea. Ten sensory panelists, who are all specialists in tea science at Huazhong Agricultural University and professionally trained in sensory evaluation, evaluated the sensory characteristics of the wet fermented samples according to the scoring criteria (Suppl. 1). The sensory evaluation was conducted in triplicate.

2.4. Determination of pH value

The pH value of pickled tea was precisely measured by an electronic pH meter (FE20 type, made in Shanghai, China). Ten grams of tea samples were added into 10 mL of distilled water, and then the sample was completely homogenized for approximately 20 min by a disintegrator (Jiuyang Juicer JYL-B060; Joyong Inc., China). The mixed homogenate was filtrated by double-layer cotton gauze and the filtrate was used to measure the pH value.

2.5. Determination of common components and tea pigments

The common components of the samples, including tea polyphenols (TPs), FAAs, soluble sugar, caffeine, and flavonoids were determined by using a UV–Vis spectrometer (Zhou, Chen, & Ni., 2009), and the soluble extract of the dried leaf samples was determined according to the drying method (Zhou et al., 2009).

The tea pigments (theaflavins, TFs; theabrownines, TBs; and thearubigins, TRs) were analyzed using the system analysis method (Yao et al., 2006). The sample at 3.0 g was extracted with 125 mL boiling distilled water in a boiling water bath for 10 min with shaking (1–2 times) and subsequently filtered. The tea filtrate was rapidly cooled to room temperature. The tea filtrate (30 mL) was shaken with 30 mL of ethyl acetate extract (EtOAc) for 5 min, the layers were separated after equilibration. A sample of the EtOAc layer (2 mL) was diluted to 25 mL with 95% ethanol (solution A). The 15 mL EtOAc layer was mixed with 25 mL of $NaHCO_3$ solution (2.5 g/100 mL) for 30 s, and a 4 mL sample of the regenerated EtOAc layer was diluted to 25 mL with 95% ethanol (solution C). A 2 mL sample of the aqueous layer (first) was mixed with 6 mL distilled water and 2 mL saturated oxalic acid solution and then diluted to 25 mL with 95% ethanol (solution D). Tea filtrate (15 mL) was mixed with 15 mL of butyl alcohol for 3 min, and the layers were separated after equilibration. Then, a 2 mL sample of the aqueous layer (second) was mixed with 2 mL of saturated oxalic acid solution and 6 mL of distilled water and diluted to 25 mL with 95% ethanol (solution B).

The optical densities (*E*A, *E*B, *E*C and *E*D) of solutions A, B, C and D, respectively, were measured in a 1 cm cell at 380 nm using a spectrophotometer (Model-755B, Shanghai Optical Instrument Factory). Total concentrations of TFs, TRs, and TBs were calculated from the following equations: