JOURNAL OF FOOD AND DRUG ANALYSIS 23 (2015) 709–718

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Effects of blending wheatgrass juice on enhancing phenolic compounds and antioxidant activities of traditional kombucha beverage

Tzu-Ying Sun, Jia-Shiun Li, Chinshuh Chen *

Department of Food Science and Biotechnology, National Chung Hsing University, Taichung, Taiwan

ARTICLE INFO

Article history:
Received 15 September 2014
Received in revised form
16 January 2015
Accepted 27 January 2015
Available online 27 March 2015

Keywords:
absorbance capacity
antioxidant activity
kombucha
oxygen radical phenolic compounds
wheatgrass juice

ABSTRACT

Traditional kombucha is a fermented black tea extract and sugar. Sweetened black tea (10% w/v) and wheatgrass juice (WGJ) were mixed in various ratios and used as fermentation substrate for enhancing phenolic compounds and antioxidant activity. Starter, comprising of yeast (*Dekkera bruxellensis*) and acetic acid bacteria (*Gluconacetobacter rhaeticus* and *Gluconobacter roseus*), was inoculated at 20% (v/v), and fermented statically at 29 ± 1°C for 12 days. The results showed that the total phenolic and flavonoid contents and antioxidant activity of the modified kombucha were higher than those of traditional preparations. All WGJ-blended kombucha preparations were characterized as having higher concentrations of various phenolic compounds such as gallic acid, catechin, caffeic acid, ferulic acid, rutin, and chlorogenic acid as compared to traditional ones. Addition of WGJ resulted in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging ability of kombucha being > 90%, while the oxygen radical absorbance capacity increased from 5.0 μmol trolox equivalents/mL to 12.8 μmol trolox equivalents/mL as the ratio of WGJ increased from 0% to 67% (v/v). The highest antioxidant activity was obtained using a 1:1 (v/v) black tea decoction to WGJ ratio and 3 days of fermentation, producing various types of phenolic acids. These results suggest that intake of fermented black tea enhanced with wheatgrass juice is advantageous over traditional kombucha formulas in terms of providing various complementary phenolics and might have more potential to reduce oxidative stress.

1. Introduction

Kombucha is a well-known fermented health beverage popular in many countries [1]. Kombucha is traditionally made by fermenting sugared black tea using a symbiotic culture of acetic acid bacteria (e.g., *Acetobacter xylinum*, *Acetobacter xylinoides*, or *Bacterium gluconicum*) and yeast (e.g., *Schizosaccharomyces pombe*, *Saccharomyces ludwigii*, *Zygosaccharomyces rouxii*, *Candida* spp., or *Pichia* spp.) statically for 2 weeks. The

* *Corresponding author*. Department of Food Science and Biotechnology, National Chung Hsing University, 250 Kuokuang Road, Taichung 402, Taiwan.
E-mail address: cschen@mail.nchu.edu.tw (C. Chen).
http://dx.doi.org/10.1016/j.jfda.2015.01.009

fermentation product is comprised of two components: a floating cellulosic pellicle layer and a sour-tasting and slightly sparkling liquid broth [2]. It contains many compounds with antioxidant activity, such as phenolics, water-soluble vitamins, organic acids, and minerals [2,3]. Williamson et al [4] demonstrated that the antioxidant activity of kombucha, which may be attributed to the tea polyphenols, can increase the *in vivo* antioxidant ability. Oral administration of kombucha to rats exposed to pro-oxidation species also indicated the potent antioxidant properties of the fermented drink such as decrease of the degree of lipid oxidation and DNA fragmentation [5].

The antioxidant activity of kombucha corresponds highly to the fermentation substrate (tea leaves); moreover, some components of tea promote cellulose formation from acetic acid bacteria [6]. Greenwalt et al [7] showed that using green tea as the substrate could minimize the fermentation time; however, to retain its characteristic flavor, black tea is still used as the fermentation substrate in the traditional kombucha [6].

Substances with antioxidant properties offer many benefits to the human body [8]. The antioxidants found in kombucha fermentation substrates originated in tea leaves and mainly include polyphenols, especially catechins, which belong to the flavones group [5,9]. Black tea leaves in substrates used for preparing traditional kombucha account for only 1% (w/v) of the total, and in fact do not play a major role in fermentation compared with sucrose (ca. 10%, w/v). Instead, black tea contributes the sensory attributes such as flavor and taste to kombucha, and with a lesser extent on acting as a source of antioxidant substances. The beneficial effects, particularly in antioxidant activity and phenolic substance content, would be further enhanced if we supplement the traditional kombucha with other substrates such as herbs or vegetables.

Wheatgrass juice (WGJ) is an extract squeezed from the mature sprouts of wheat seeds (*Triticum aestivum*). The therapeutic qualities of WGJ have been attributed to its rich nutrient contents, including chlorophyll, vitamins (A, C, and E), bioflavonoids, minerals (iron, calcium, and magnesium), and phenolics (ferulic acid and vanillic acid) [8]. Kulkarni et al [10] reported that WGJ has high antioxidant activity partly because it contains such antioxidants as phenolic compounds and several flavonoids. Phenolics and flavonoids have been shown to remove superoxide radicals (O_2^- or HO_2^-) *in vivo* and decrease the cell damage caused by oxidative stress [9]. Wheatgrass extracts also possess superoxide scavenging and ferric reducing abilities [8,10]. Their ability to inhibit oxidative DNA damage was also demonstrated [11].

Several days of acetic acid bacteria and yeast co-culture have been shown to yield a high antioxidant activity. However, the fermentation process is long and produces a large amount of acetic acid from acetic acid bacteria, which affects the flavor of the drink. Therefore, long fermentation processes are not suitable for health beverage production. In addition, few studies have used materials besides tea leaves as the fermentation substrate for kombucha. The aim of this study is to assess the changes in kombucha's antioxidant activity and phenolic compounds during fermentation as affected by different ratios of sugared black tea decoction and WGJ.

2. Methods

2.1. Starter culture

Starter culture, or kombucha culture, was collected from a local cultivator who grows kombucha periodically in Taichung, Taiwan and maintained in sugared black tea. The culture includes both the upper pellicle layer and the lower liquid. The major bacterial components were identified as *Gluconacetobacter rhaeticus* and *Gluconobacter roseus* and the yeast component as *Dekkera bruxellensis* at Mission Biotech (Taipei, Taiwan). The starter culture was periodically maintained according to method of Chen and Liu [2], except that black tea leaves were used.

2.2. Sweetened black tea

Ten percent (w/v) sucrose was added to deionized water and heated at 100°C for 5 minutes. Next, 1% (w/v) black tea leaves (Ten-Ren Co., Taipei, Taiwan) were added, allowed to steep for 15 minutes, and then filtered through a sterile sieve.

2.3. WGJ

The wheatgrass (*Triticum aestivum* L.) was purchased from the Santa Cruz supermarket, Taichung City, Taiwan and washed with tap water and steam-blanched (100°C, 30 seconds). WGJ was extracted with a juicer (Green Power, KP-E1201; Kempo Co. Ltd., Seoul, Korea).

2.4. WGJ-blended kombucha fermentation

Sweetened black tea and WGJ were mixed in various ratios to create six groups of WGJ-blended black tea broth, with five glass jars (capacity 500 mL) in each group (Table 1). To each group, the broth was dispensed equally into five glass jars (each containing 120 mL broth) that had been previously sterilized at 121°C for 20 minutes. Each jar was then inoculated with 30 mL of previously fermented kombucha liquid starter that had been cultured in the sweetened black tea for 10 days. The jars were carefully covered with clean cheesecloth and fastened with rubber bands. The fermentation was

Table 1 – Combinations of black tea decoction and wheatgrass juice as fermentation substrates for preparing WGJ-blended kombucha.

Component	Group					
	TK	WK1	WK2	WK3	WK4	WK5
Black tea decoction (mL)[a]	120	80	60	40	0	0
Wheatgrass juice (mL)	0	40	60	80	120	120(S)[c]
Starter (mL)[b]	30	30	30	30	30	30
Total volume (mL)	150	150	150	150	150	150

TK = traditional kombucha; WK1–5 = kombucha supplemented at various ratios with wheatgrass juice.
[a] Black tea decoction contains 10% (w/v) sucrose.
[b] Starter: Freshly fermented kombucha (lower liquid portion, 30 mL) was used as inoculum or starter.
[c] Wheatgrass juice contains 10% (w/v) sucrose.