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Green tea (*Camellia assamica*) concentrate as a source of L-theanine used in kombucha fermentation for relaxation drink[1)]

Konsentrat teh hijau (Camellia assamica) *sebagai sumber L-theanine yang digunakan pada fermentasi kombucha untuk minuman relaksasi*

Agustine SUSILOWATI[*)], ASPIYANTO, Hakiki MELANIE & Yati MARYATI

Research Center for Chemistry, Indonesian Institute of Sciences,
Kawasan PUSPIPTEK, Serpong, South Tangerang 15314

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Abstrak

Konsentrat teh hijau (Camellia assamica) grade *Pekoe yang dihasilkan melalui pemurnian oleh membran mikro-filtrasi (MF) berpotensi sebagai substrat pada fermentasi kombucha untuk efek relaksasi (anti stres) karena kandungan L-theanine yang tinggi. Kegiatan penelitian dilakukan untuk mendapatkan ratio terbaik antara konsentrat teh hijau dan air guna menghasilkan komposisi teh kombucha yang setara dengan teh kombucha komersial sebagai minuman santai. Fermentasi dilakukan pada nisbah konsentrat teh hijau dan air 1:4, 1:3, 1:2, 1:1 dan 1: 0 (v/v) dengan starter Kombucha komersial 0,5 % (b/v) pada suhu kamar selama satu minggu. Sebagai kontrol digunakan teh kombucha dengan nisbah teh hijau komersial dan air adalah 1:0. Hasil penelitian menunjukkan bahwa kandungan L-theanine dalam produk teh kombucha yang setara dengan produk komersial (2,51 % berat kering) dicapai pada nisbah konsentrat teh hijau dan air 1 : 2 (2,16 % berat kering) dan 1:1 (3,01 % berat kering). Selain L-theanine, pada ratio konsentrat teh hijau dan air tersebut teh kombucha yang dihasilkan mengandung total polifenol 1,03 %, total padatan 11,67 %, total asam 0,53 % dan gula pereduksi 27,4 mg/mL, serta jumlah mikroba total log 5,88 CFU/mL atau 7,72 x 10^5 CFU/mL.*

[*Kata kunci*: Camellia assamica, *kombucha, membran mikrofiltrasi, teh hijau, L-theanine*]

Abstract

Concentrate of green tea (*Camellia assamica*) of *Pekoe* grade produced through purification by means of microfiltration (MF) membrane is potentially used as a substrate in kombucha fermentation for relaxation (anti stress) due to high L-theanine concentration. This experiment was conducted to find out the best ratio of green tea concentrate and water in order to generate kombucha tea composition that is equal to commercially available kombucha tea as relaxation drink. Fermentation process was performed with green tea concentrate and water ratio of 1: 4, 1: 3, 1: 2, 1: 1 and 1: 0 (v/v) using 0.5 % (w/v) of commercial kombucha starter at room temperature for one week. As a control kombucha tea with ratio of commercial green tea and water of 1 : 0 was used. The result shows that L-theanine content in kombucha tea product equal to commercial kombucha tea (2.514 %, dry weight) was reached at the ratio of green tea concentrate and water of 1 : 2 (2.16 %, dry weight) and 1 : 1 (3.01 %, dry weight). Beside L-theanine, kombucha tea resulted from this ratio of green tea concentrate and water contains total polyphenol 1.03 %, total solids 11.67 %, total acids 0.53 % and reducing sugar 27.4 mg/mL, and total microbial counts of log 5.88 CFU/mL or 7.72 x 10^5 CFU/mL.

[Keywords: *Camellia assamica*, kombucha, microfiltration membrane, green tea, L-theanine]

Introduction

L-theanine is a unique amino acid component analog glutamine found only in leaves, branch, stem, the whole parts of tea tree (*Camellia sinensis*), green tea (*Camellia assamica*), and several fungi such as *Cunninghamella echinulata* (Jiayou *et al*., 2006). L-theanine is generally considered one of the most effective substances for anti stress (relaxation). This component is able to stimulate production of wave in brain (0.5 – 3 Hz), and to increase dopamine and serotonine concentrations. It has an important role in the formation of neurotransmitter of gamma-aminobutyric acid (GABA) in order to give relaxation sense without causing nervous sense (Xiao, 2006; Adham *et al*., 2006; Liu, 2006a).The chemical structure of L-theanine or N_γ-Ethyl-L-glutamine or L-Glutamic acid γ-(ethylamide) or $C_7H_{14}N_2O_3$ is shown in Figure 1. It has been known that L-theanine content is not only influenced by variety and grade, but also by processing technique. Local green tea of *Pekoe* grade is commonly used for ready-to-drink with fan firing method (Anonymous, 2007). The use of this green tea in extraction and purification of L-theanine as a functional compound in kombucha fermentation will increase its economic value.

L-theanine can be separated and purified through MF membrane. MF system is chosen because the separation process is based on differences in molecular weight (MW) and particle size of components (Susilowati *et al*., 2009). MF membrane of 0.2 µm at the best condition of process (pump motor frequency of 20 Hz, room temperature and operation pressure of four bar for 120 min) will generate concentrate (retentate), which is components retained on membrane surface with particle size > 0.2 µm, and permeate,

*) Corresponding author Email: agustine_408@yahoo.com Phone: +62-21-7560929

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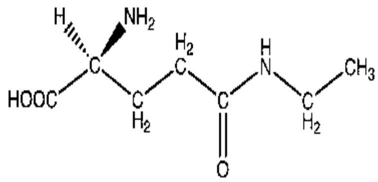


Figure 1. L-theanine N_γ-Ethyl-L-glutamine or L-Glutamic acid γ-(ethylamide) or $C_7H_{14}N_2O_3$.

Gambar 1. L-theanine N_γ-Ethyl- L-glutamine atau asam L-Glutamic γ-(ethylamide) atau $C_7H_{14}N_2O_3$.

components with particle size <0.2 µm passing through membrane pores. L-theanine has MW of 174 Da (particle size of 0.001 – 0.1 µm), therefore it found more in permeate (Anonymous, 2005). Permeate is used as a raw material of anti stress medicine, while concentrate (retentate) is a functional drink material for relaxation.

The concentrate is used as substrate in kombucha fermentation to get better functional properties compared to general kombucha. Kombucha starter cultures are symbiotic relationship between yeast/fungi and microbe (acetic acid bacteria), and are gelatinous and though membrane of fungi cultures, flat disc shape containing acetic acid bacteria (*Acetobacter xylinum*), yeast (*Saccharomyces cereviseae*, *Saccharomyces ludwigii*, *Saccharomyces bisporus*, *Zygosaccharomyces* sp.) and several yeast types (*Torolupsis* sp.) (Malbasa *et al*., 2008). Fungi cell will hydrolyze sucrose to glucose and fructose in order to produce ethanol, whereas bacteria will convert glucose to gluconic acid, and fructose will form acetic acid. *Acetobacter* sp. as main bacteria in kombucha cultures oxidize ethanol to acetaldehyde and then to acetic acid. The culture contains glucuronic acid, lactic acid, vitamins, amino acids, antibiotic, and other useful materials for health (Jayabalan *et al*., 2008), and generates specific aroma drink of kombucha analogue to fresh fruit aroma or acids or vinegar (Dufresne & Farnworth, 2000) through assimilative and dissimilative chemical reactions during 7–14 days of fermentation.

The objective of this experiment was to determine the optimum ratio of green tea concentrate and water as a fermentation substrate to produce kombucha tea with the best L-theanine concentration equal to commercial kombucha tea.

Materials and Methods

Materials and equipment

Materials used in this experiment were local dry green tea (*Camellia assamica*) of grade *Pekoe* purchased from Perkebunan Teh Gambung Argo Lestari, Gambung, West Java, commercial kombucha starter culture/colony, chemical reagent of analytical grade quality for analysis, and commercial polysulfone microfiltration (MF) membrane of 0.2 µm with 0.036 m^2 of effective area (GRM-0.2-PP, Danish Separation Systems, Denmark).

Main equipment utilized in this work were extractor in semi pilot scale (15 – 25 L), heater, filtration (High Frequency Separation), cation exchange resin column, plate and frame type cross-flow membrane filtration module unit type DSS LabUnit M20, Denmark with adjustable membrane area equipped with high pressure cross-flow pump of Positive Displacement type Rannie 25.38 with motor (3 – 15 L/min. at max. 60 bar) (Anonymous, 2000), fermentation equipment system in laboratory scale, and Spectrofotometer UV-1201.

Research design

Kombucha preparation from concentrate of local green tea of *Pekoe* grade was conducted through kombucha tea fermentation with a variation of green tea concentrate and water ratio of 1 : 4, 1 : 3 , 1 : 2, 1 : 1 and 1 : 0 using commercial kombucha starter culture (5 %, v/v) and sucrose (10 %, w/v) at room temperature for one week. Green tea concentrate was obtained through extraction, filtration via a 80 mesh sieve, filtration via a 200 mesh sieve, removal of metal, and purification by 0.2 µm MF membrane with pump motor at frequency of 20 Hz, room temperature and operation pressure 4 bar for 120 min. Analysis was carried out on feed, result of MF membrane (permeate and concentrate), and kombucha tea product for each ratio, covering total solids (Gravimetric), reducing sugar (Somogyi-Nelson), total acids (Titration) (AOAC, 1980), L-theanine (Ninhydrin method) (Xiao, 2006), total polyphenol (Folin-Denise method) (Liu, 2006b), and total Plate Count (Pour plate method) (Fardiaz, 1989).

Extraction process of green tea

Extraction process was carried out by adding 1 kg dry granular green tea to 8 L fresh water at ± 90ºC for 15 min, agitated for 5 min and allowed to immerse for 15 min and then filtered through a 80 mesh sieve to obtain filtrate (I) and residue (I). One part of residue (I) was re-extracted by adding seven parts of hot fresh water (± 90ºC) for 15 min and filtered through a 80 mesh sieve to separate filtrate (II) and residue (II). Filtrate (I) and (II) were mixed to obtain green tea extract. Green tea extract was filtered by means of High Frequency Separation using a 200 mesh sieve to separate the filtrate and residue (Susilowati *et al*., 2009). The filtrate was used in separating process of amino acids as functional compound through 0.2 µm MF membrane.

Microfiltration process

Green tea extract placed in 9 L of feed tank with overflow was pumped by Positive Displacement Pump through a 200 μm filter, a heating/cooling water system, and a cross-flow MF membrane module to separate permeate and concentrate. The concentrate was circulated into the feed tank after measuring the flow rate to keep the process stable. During the circulation process, tap water in chiller (± 23–24°C) was flowed into heating/cooling water system until fluid temperature in feed/concentrate tank was constant at room temperature (25°C). After the process was stable, pump motor frequency was adjusted at 20 Hz (Flow rate of feed fluid was kept at 7.5 L/minute). The mean value of pressure gauges at the inlet and outlet of the membrane module was taken as the operating pressure, and kept constant at four bar (Anonymous, 2000). Fluid passing via membrane pores area expressed in terms of permeate was collected into a mass cylinder in order to determine permeate flux value. Sampling was done on permeate and concentrate for 120 min.

Kombucha fermentation

Kombucha starter culture was prepared by inoculating a small amount of kombucha starter on Potato Dextrose Agar medium and incubated at 30°C for five days. Kombucha starter liquid was prepared by pouring 100 mL of sterilized water to cultured kombucha and shaked slowly to form suspension containing microbes. Suspension of kombucha starter (5 %, v/v) was added to biomass of green tea concentrate that had been previously added sucrose (10 %, w/v) and sterilized at 121ºC for 15 min. Fermentation was done in a dark beaker glass at room temperature for one week. The whole activities were aseptically done in a laminar air flow cabinet. Sampling was done by taking one bottle from the incubator after one week of culture. Samples were used for the measurement of microbial population and chemical changes. Schematic diagram of preparation process of kombucha tea from green tea of *Pekoe* grade is shown in Figure 2.

Results and Discussion

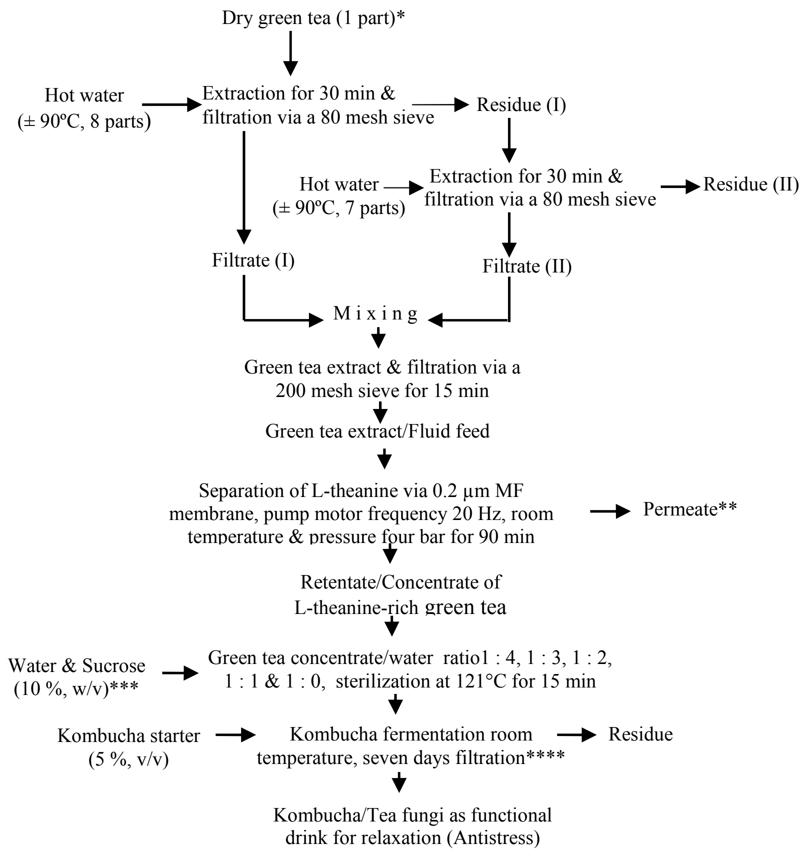
Characteristics of dry leaves and extract of Pekoe grade green tea

Green tea leaf of *Pekoe* grade is ball roll like, short, less uniform, rather hard texture, less elastic, and grayish green color. Preparation process with high temperature (100-175ºC) for 2 h (Anonymous, 2007) may cause darker leaf color as a consequence of Maillard reaction (Belitz & Grosch, 1999). Extraction process of the green tea is performed to obtain feed, which will be used in separation of L-theanine. This extraction process resulted in a rather thick, dark green to brownish dark yellow with specific aroma of green tea, astringent and less bitter taste, and with 12.42 % L-theanine, 5.8 mg/mL dissolved protein, and 4.83 % total solid. This composition is higher than L-theanine content of dry leaves (1,5 – 3 %) or 50 – 70 % of total free amino acids (Jiayou *et al*., 2006; Liu, 2006b). Figures 3a and 3b show dry green tea of *Pekoe* grade and suspension of green tea extract. The high content of L-theanine and dissolved protein were not only caused by green tea leaves factors, such as variety, location, processing method (fan firing), but also by extraction and separation process.

Particle size of amino acids ranges between 0.01 – 0.1 μm, so that they pass as filtrate. High solubility property of L-theanine enable also to get better extraction of L-theanine. Dissolved protein is all dissolved peptides and amino acids in water, in which its presence is influenced by treatment process of tea. Total solids content after filtration through 200 mesh sieve is yielded total solids content of 4.83 %, in which filtration process separates succesfully >25 % of component (Susilowati *et al.,* 2009). Dry matter of green tea plays an important role in performance of MF membrane on separation components in green tea due to directly affect flow rate of material, which will influence recovery of L-theanine in permeate and retentate.

Effect of process condition of MF on retentate and permeate compositions of green tea

Separation process of L-theanine by means of MF membrane 0.2 μm at pump motor frequency of 20 Hz, at room temperature and operation pressure of four bar for 120 min resulted retentate, suspension retained on membrane surface as thick brown liquid with astringent and less bitter, and permeate, clear brown liquid with astringent and less savory. Figure 4 displays permeate as green tea extract and retentate (concentrate) as a result of L-theanine separation through MF membrane. Retentate consists of L-theanine of 5.74 % (dry weight), dissolved protein of 26 mg/mL, total solids of 2.57 % and total polyphenol of 17.12 %, while concentrations of L-theanine, dissolved protein, total solids and total polyphenol in permeate were 10.72 % (dry weight), 21mg/mL, 1.31% and 26.1 %, respect-tively. This difference is not only caused by driving force in MF system at operation pressure of four bar, but also is caused by interaction between all process treatment and material composition. L-theanine accumulated more in permeate due to its particle size less than 0.2 μm (0.01 – 0.1 μm) (Anonymous, 2005). This condition occurred for polyphenol compounds as well. Whereas, more amount of total solids and dissolved protein were obtained in retentate because their particle size are more than 0.2 μm. Total solids are the accumulation of all components of green tea forming cake layer on membrane surface or fouling, therefore they are difficult to pass the membrane, although their particle size are less than 0.2 μm. Table 1 demonstrates components composition of retentate and permeate from MF result on green tea extract of *Pekoe* grade.



Note : * *Camellia assamica* of *Pekoe* grade via fanfiring process,
** as green tea extract for raw material of antistress medicine,
*** each treatment for green tea concentrate/water ratio
**** separation of kombucha colony.

Figure 2. Schematic diagram of preparation process of kombucha tea fungus from concentrate as a result of separation L- theanine from green tea of *Pekoe* Grade by 0.2 µm MF membrane for functional drink of antistress.

Gambar 2. Diagram skematik proses pembuatan teh kombucha dari konsentrat hasil pemisahan L-theanine dari teh hijau grade Pekoe oleh membran MF 0,2 µm untuk minuman fungsional anti stres.

Effect of fermentation process of green tea concentrate on kombucha tea composition L-theanine and total microbial count

Fermentation process at the low ratio of green tea concentrate and water for seven days showed an increase of L-theanine concentration, and optimum condition was reached at the ratio of green tea concentrate and water of 1:0 (Figure 5). Total microbial counts gradually increased at the ratio of 1 : 2 then decreased at ratio of 1 : 0 (Figure 5). Ratio of green tea concentrate and water of 1:4, 1 : 3, 1 : 2, 1 : 1 and 1 : 0 in this fermentation process give L-theanine concentration of 1.22, 1.49, 2.16, 3.01 and 4.45% (dry weight), and L-theanine concen-tration in kombucha tea as control of 2.51 % (dry weight). Raising of L-theanine concentration at low ratio of green tea concentrate and water is caused by adding less water to lower L-theanine concentration in suspension. One week of fermentation process shows a difference in L-theanine recovery before (5.74 % dry weight) and after fermentation process (4.45 % dry weight) at ratio of 1: 0 or reduction by 22.54 %. L-theanine was used by microorganism for their growth or reactions relating with microbial metabolism. Based on functional properties of L-theanine as relaxation compound, L-theanine content in kombucha tea product equal to commercial kombucha tea (2.51 %, dry weight) was reached at the ratio of green tea concentrate and water of 1 : 2 (2.16 %, dry weight) and 1 : 1 (3.01 %, dry weight). Kombucha fermentation at room temperature (± 26 – 28ºC) for seven days resulted brown clear liquid with specific aroma of kombucha tea. Kombucha colony was appeared on one L-beaker glass surface like gelatinous layer and dish-

Figure 3. Dry green tea of *Pekoe* grade via pan firing process (a) and green tea extract as feed in separation process of L-theanine using MF membrane (b).

Gambar 3. Teh hijau kering grade Pekoe melalui proses pan firing (a) dan ekstrak teh hijau sebagai umpan pada proses pemisahan L-theanine menggunakan membran MF (b).

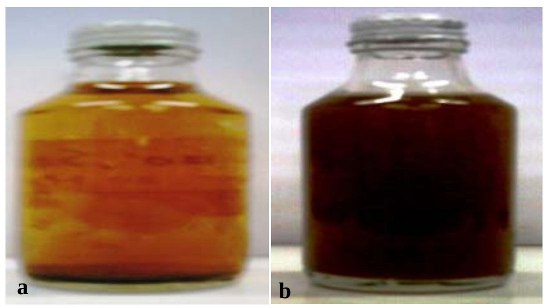


Figure 4. Permeate (a) and retentate (concentrate) (b) as a result of separation process of L-theanine from green tea of *Pekoe* grade through 0.2 µm MF membrane at pump motor frequency of 20 Hz, room temperature and operation pressure of 4 bar for 120 min.

Gambar 4. Permeat (a) dan retentat (konsentrat) (b) hasil proses pemisahan L-theanine dari teh hijau grade Pekoe melalui membran MF 0,2 µm pada frekuensi motor pompa 20 Hz, suhu ruang dan tekanan operasi 4 bar selama 120 menit.

Table 1. Compositions of retentate and permeate as a result of MF of green tea of *Pekoe* grade.

Tabel 1. Komposisi retentat dan permeat hasil MF terhadap teh hijau grade Pekoe.

Material type *Jenis bahan*	Components (*Komponen*)			
	L-theanine (% dry weight) (% *berat kering*)	Total polyphenol (% dry weight) *Jumlah polipenol* (% *berat kering*)	Dissolved protein *Protein terlarut* (mg/mL)	Total solids *Padatan total* (%)
Retentate (Concentrate) *Retentat (Konsentrat)*	5.74	17.12	26	2.57
Permeate (Extract) *Permeat (Ekstrak)*	10.72	26.10	21	1.31

like plate. Colony growth was different in each ratio of with green tea concentrate and water. The colony growth flatness level on glass surface and the best gelatinous thickness were reached at the ratio of green tea concentrate and water of 1 : 3. As had been seen that fungus tea is a symbiotic amongst fungi, acetic acid bacteria and less yeast (Teoh *et al*., 2004). On its growth in Potate Dextrose Agar medium, colony of acetic acid bacteria is more dominant when compared with fungi, so that microbial count is total bacteria and fungi. Low ratio of green tea concentrate and water will increase total microbial count to reach optimal microbial count followed by declining them at the lowest ratio of green tea concentrate and water (1 : 0). At ratio of green tea concentrate and water of 1:4, 1:3, 1: 2, 1:1 and 1:0, total microbial count are log 5.34, log 5.67, log 5.93, log 5.89 and log 5.75 CFU/mL, respectively, and total microbial count in kombucha tea as control is log 5.75 CFU/mL. Increase of total microbial count related to the

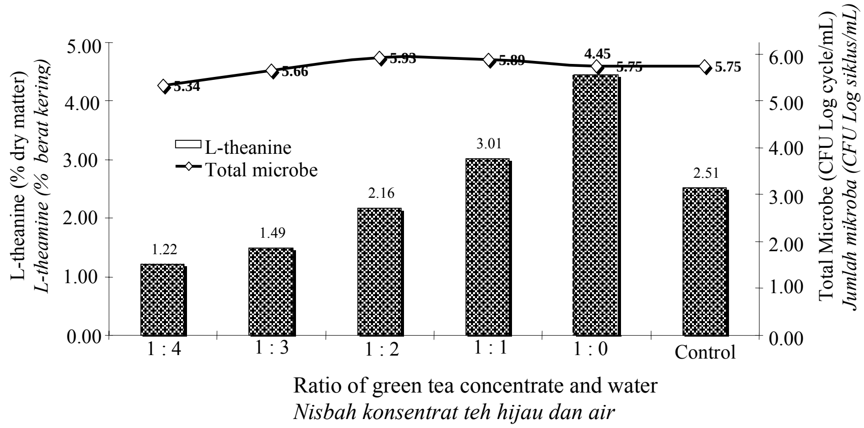


Figure 5. Effect of the ratio of green tea concentrate and water on L-theanine concentration and total microbial counts in kombucha tea as a result of fermentation of local green tea concentrate at room temperature for one week.

Gambar 5. Pengaruh rasio konsentrat teh hijau dan air terhadap konsentrasi L-theanine dan jumlah mikroba total pada teh kombucha hasil fermentasi konsentrat teh hijau lokal pada suhu ruang selama satu minggu.

addition of sucrose (10 %, w/v) for each treatment as sucrose becomes main source of carbon on the growth of microbes. Low ratio of green tea concentrate and water will provide much higher sucrose concentration due to low dilution level in order to grow microbes. This matter is also supported by similar kombucha starter (5 %, v/v) in order to raise total microbial count to optimal ratio of green tea concentrate and water. This condition is reached at ratio of green tea concentrate and water of 1: 2 with total microbial count of log 5.93 CFU/mL followed by dropping total microbial count at ratios of green tea concentrate and water of 1:1 and 1:0, namely log 5.89 and log 5.75 CFU/mL. This condition is possibly caused by less carbon source or lysis due to anti microbe agent from green tea components. Polyphenol plays an important role as anti microbe on *Helicobacter pylori* of gastritis promoter, *E. coli*, *S. aureus* and *Agrobacterium tumefaciens*. Interaction between microbe growth and green tea concentrate decreases the total microbial count, especially on bacteria by polyphenol inhibiting growth of pathogenic bacteria in intestinal, but it has no effect on Lactic Acid Bacteria (LAB) (Greenwalt *et al.,* 1996).

Total solids and reducing sugar

Kombucha fermentation for seven days at decreasing ratio of green tea concentrate and water produced kombucha tea with high concentration of total solids, while at similar process treatment yielded fluctuated concentration of reducing sugar followed by raising concentration of reducing sugar at the lowest ratio of green tea concentrate and water (1:0), as illustrated in Figure 6. Ratio of green tea concentrate and water of 1:4, 1:3, 1:2, 1:1 and 1: 0 in Kombucha fermentation generate total solids of 11.59 %, 11.27 %, 11.23 %, 11.67 % and 12.34 % and in kombucha tea as control of 13.59 %. Compared with initial total solids concentration before fermentation (2.57 %), kombucha fermentation process increases total solids for all ratios of green tea concentrate and water. This increase might be due to sucrose contribution (10 %, w/v) and kombucha cultures (0.5 %, v/v), and metabolism result of kombucha cultures. All ratios of green tea concentration and water indicate lower total solid concentration in kombucha tea product than that in control kombucha tea using commercial green tea as raw material (13.59 %). It indicates that initial composition and kind of material affect composition recovery after fermentation process. Concentrate contains solids with uniform size of particle (> 0.2 μm), whereas control with no uniform size of solid particle with size of component particle <0.2 μm to produce higher total solids concentration.

Reducing sugar concentration in kombucha tea is sucrose from fermentation process. In this kombucha fermentation, sucrose is source of carbon to form ethanol, acetaldehyde and organic acids affected by starter amount, environmental condition and enzyme activity of invertase. In other words, sucrose is also used by bacteria *A. xylinum* to form cellulose as ‘nata/pelikel‘ and suspension on medium surface (Anonymous, 2008). With ratio of green tea concentrate and water of 1:4, 1:3, 1:2, 1:1 and 1:0, reducing sugar concentration in kombucha tea product were 29.6, 27.4, 29.6, 27.4 and 36.8 mg/mL, respectively, and reducing sugar concentration in Kombucha tea as control of 25.2 mg/mL. This Kombucha fermentation process produced fluctuated concentration of reducing sugar at ratio of green tea concentrate and water of 1 : 4, 1: 3, 1 : 2 and 1 : 1, and reducing sugar concentration higher at ratio of green tea concentrate and water of 1:0. This is possibly caused by using sucrose amount (10 %, w/v), but at

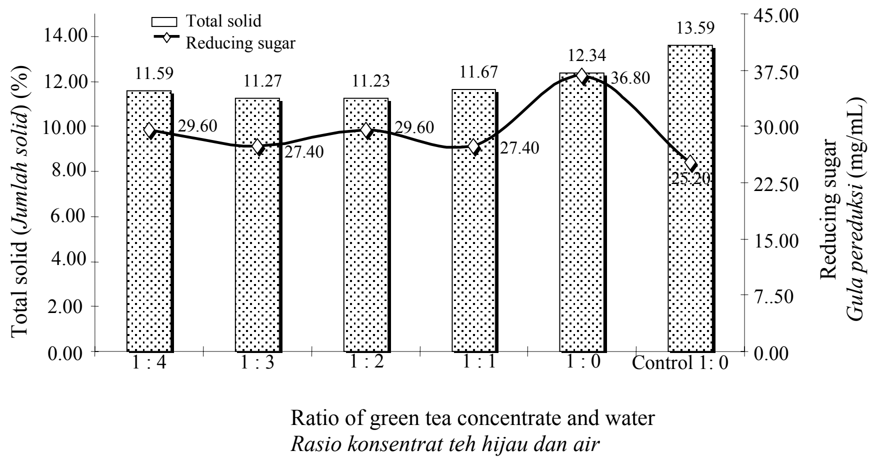


Figure 6. Effect of the ratio of green tea concentrate and water on total solids and reducing sugar concentrations in kombucha tea as a result of fermentation of local green tea concentrate of *Pekoe* grade at room temperature for one week.

Gambar 6. Pengaruh rasio konsentrat teh hijau dan air terhadap konsentrasi padatan total dan gula pereduksi pada teh kombucha hasil fermentasi konsentrat teh hijau lokal pada suhu ruang selama satu minggu.

ratio of green tea concentrate and water becoming lower due to distribution of carbon source to metabolite microbe becomes different result because of similar composition of microbes addition (0.5 %, v/v). This matter causes ability of microbes in order to adsorp sucrose to form metabolite. Difference in recovery of reducing sugar from green tea concentrate at ratio green tea concentrate and water of 1 : 0 with control Kombucha tea at similar ratio (25.2 mg/mL) indicated that green tea concentrate has much richer nutrition because it remains higher reducing sugar (36.8 mg/mL).

Kombucha fermentation for seven days at decreasing ratio of green tea concentrate and water generates high concentrations of total polyphenol and total acids, as illustrated in Figure 7. With ratio of green tea concentrate and water of 1:4, 1: 3, 1 : 2, 1: 1 and 1 : 0 gave total polyphenol concentration of 0.45, 0.58, 0.79, 1.03 and 1.86 %, respectively, and total polyphenol concentration in kombucha tea product as control at ratio of green tea concentrate and water of 1:0 was 0.56 %. Green tea concentrate is yielded from MF system, in which polyphenol as main component of green tea with particle size of 0.01 – 0.1µm and molecular weight of 300–600 Da will be trapped in cake layer due to its occurrence of fouling during MF process (Susilowati *et al*., 2009), so that its total polyphenol concentration is higher than green tea extract without MF process. Total polyphenol concentration at ratio of green tea concentrate and water 1: 0 dropped from 17.13 % (before fermentation) to 1.86 % (after fermentation) for seven days or reduction of 89.16 %. This drop is caused by its occurrence of total polyphenol degradation to organic acids, acetaldehyde, alcohol and CO_2 in order to specific kombucha tea flavor. This mechanism is caused by activity of katalase enzyme from fungi, such as *Candida tropicalis* as pre-dominant factor. Tea polyphenol has a high antioxidant property because it can inhibit free radical and activated oxygen, such as free radical of superoxyde and free radical of hydroxyl. Green tea Polyphenol/Cathecins present in green tea, such as 10 – 15 % or 25 – 40 % consisted of complex cathecin, namely EGCG, GCG, ECG, ECG, GC, EC, and C, in which each component has a different degradation rate during fermentation process (Jayabalan *et al.,* 2008).

Kombucha fermentation for seven days at decreasing ratio of green tea concentrate and water will result high concentration of total acids. Total acid is an important parameter in kombucha fermentation as metabolite produced by microbe during fermentation process (Malbasa *et al*., 2008). At ratio of green tea concentrate and water of 1: 4, 1: 3, 1: 2, 1: 1 and 1: 0, the total acid concentration were 0.33, 0.41, 0.41, 0.53 and 0.74 %, respectively, and total acid concentration in control kombucha tea at at ratio of green tea concentrate and water of 1 : 0 is 0.37 %. Increase of total acid concentration at low ratio of green tea concentrate and water indicated that microbe activity in hydrolyzing of sucrose is affected by green tea concentrate concentration and microbial count. At low ratio of green tea concentrate and water, tea polyphenol concentration becomes low, while microbe count in kombucha culture (5 %, v/v) and sucrose concentration (10 %, w/v) are fixed. At this condition, microbes will use all sucrose and tea component, especially polyphenol to form organic acids (lactic, malic, acetic and other). High polyphenol concentration will raise the formation of organic acids as total acids so that with ratio of green tea concentrate and water becoming lower (or high ratio of green tea concentrate and water) will yield organic acids as total acids with optimum

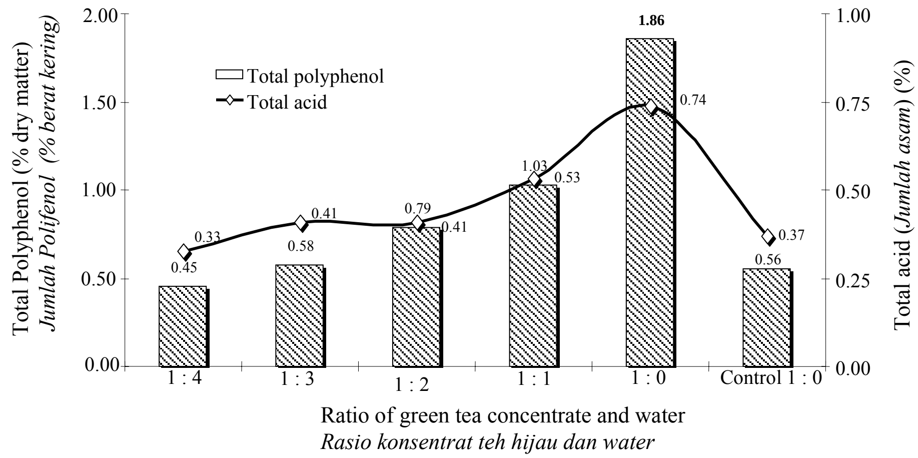


Figure 7. Effect of the ratio of green tea concentrate and water on total polyphenol and total acids concentrations in kombucha tea as a result of fermentation of local green tea concentrate of *Pekoe* grade at room temperature for one week.

Gambar 7. Pengaruh rasio konsentrat teh hijau dan air terhadap konsentrasi polifenol total dan asam total pada teh kombucha hasil fermentasi konsentrat teh hijau lokal pada suhu ruang selama satu minggu.

concentration. At this condition the pH were decreased. When compared with control kombucha tea from commercial green tea (ratio of green tea concentrate and water of 1 : 0 with total acids concentration of 0.37 %, the use of green tea concentrate at similar ratio of green tea concentrate and water generates higher concentration of total acids (49.93 %) than that in concentrate (0.74 %).

Conclusions

MF process on green tea concentrate of *Pekoe* grade generated concentrate and permeate with different composition and physical properties. The concentrate was thick suspension, yellowish brown and astringent taste, while permeate was yellowish brown clear liquid as green tea extract. Kombucha fermentation at high ratio of green tea concentrate and water would increase its composition, but optimum total microbial count was reached at ratio green tea concentrate and water of 1 : 2. The equivalent of L-theanine in kombucha tea product when compared to commercial Kombucha tea (2.51 % dry weight) was reached at the ratio of green tea concentrate and water of 1 : 2 (2.16 %, dry weight) and 1 : 1 (3.01 %, dry weight). At this ratio of green tea, kombucha tea showed concentrations of L-theanine of 3.01 %, total polyphenol of 1.03 %, total solids of 11.67 %, total acids of 0.53 % and reducing sugar of 27.4 mg/mL, and total microbial counts of log 5.89 CFU/mL or 7.72 x 10^5 CFU/mL, respectively.

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