Characteristic of kombucha tea concentrate purified through stirred microfiltration cell (Smfc) from local green tea (*Camellia sinensis* (L.) as functional drink for relaxation

Karakteristik pekatan teh Kombucha dari ekstrak teh hijau (Camellia sinensis L.*) lokal hasil Stirred microfiltration cell (Smfc) sebagai minuman fungsional untuk relaksasi*

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Abstract

L-theanine is amino acid contained in tea (Camellia sp.) leaves having ability as relaxation compound. Combination of L-theanine and other compounds produced from fermentation of green tea extract by Kombucha starter generates Kombucha tea with unique taste and better relaxation functional. The concentrate of Kombucha tea as functional drink for relaxation (anti stress) was produced by purification on fermented green te extract through 0.2 µm MF membrane fitted in stirred microfiltration cell (SMFC) at stirrer rotation speed of 200 rpm and 400 rpm, and pressure of 30 psia. Purification was conducted on 4 grades of Kombucha tea from local green tea extract with rich L-theanine, such as Pekoe, Dewata, Arraca Kiara and Arraca Yabukita as a result of purification by MF module. Kombucha tea produced through fermentation at 1 part of permeate and 1 part of water ratio using Kombucha (5%, v/v) starter riched by sucrose of 10% (w/v) under room temperature for 7 days. Based on the best L-theanine concentration, the result of experiment showed that SMFC at stirrer rotation speed of 400 rpm was able to retain L-theanine in retentate from Kombucha tea of *Arraca Kiara*, *Arraca Yabukita*, *Dewata*, and *Pekoe* grades, subsequent 29.1, 96,53, 44.62 and 71.54% (w/v) from prior to process as feed. In other words, optimization condition was reached at Kombucha tea of *Arraca Yabukita* grade producing retentate or concentrate of Kombucha tea with L-theanine, total polyphenol, reducing sugar, total solids, and total of titratable acids of 2.5605% (w/w), 3.4314% (w/w), 14 mg/mL, 4.8947% (w/w), and 0.2195% (v/v) respectively.

Keywords: Kombucha Tea, L-theanine, stirrer microfiltration cell (SMFC), flux, permeate, retentate or concentrate.

Abstrak

*L-theanine merupakan asam amino dalam daun teh (*Camellia *sp) yang memiliki kemampuan sebagai senyawa untuk relaksasi.*

Paduan L-theanine dengan senyawa-senyawa lain yang dihasilkan melalui fermentasi esktrak teh hijau menggunakan starter Kombucha akan menghasilkan teh kombuca dengan cita rasa unik dan manfaat relaksasi yang lebih baik. Untuk menghasilkan konsentrat teh Kombucha dilakukan pemurnian ekstrak teh hijau terfermentasi melalui membrane MF 0,2 µm yang dipasang pada sel mikrofiltrasi berpengaduk pada kecepatan putar 200 dan 400 rpm serta tekanan 30 psia. Pemurnian dilakukan terhadap 4 jenis teh Kombucha dari ekstrak teh hijau local kaya L-theanine yaitu Pekoe, Dewata, Arraca kiara dan Arraca yabukita hasil pemurnian melalui modul mikrofiltrasi. Teh Kombucha dihasilkan melalui fermentasi pada rasio 1 bagian permeat dengan 1 bagian air menggunakan starter Kombucha (5%, v/v) yang diperkaya dengan sukrosa 10% (w/v) selama 7 hari pada suhu ruang. Hasil penelitian berdasarkan L-theanine tertinggi menunjukkan, bahwa MF sel berpengaduk pada kecepatan putar 400 rpm mampu menahan L-theanine pada retentat teh Kombucha Arraca kiara, Arraca yabukita, Dewata dan Pekoe berturut-turut sebesar 29,1; 96,53; 44,62 dan 71,54% (w/w) dari sebelum proses sebagai feed, atau optimisasi dicapai teh Kombucha jenis Arraca yabukita yang menghasilkan retentat sebagai konsentrat teh Kombucha dengan L-theanine, total polifenol, gula reduksi, total padatan dan total asam berturut-turut sebesar 2,5605% (w/w); 3,4314% (w/w); 14 mg/mL; 4,8947% (w/w) dan 0,2195% (v/v).

***Kata kunci**:* *teh Kombucha, L-theanine, mikrofiltrasi sel berpengaduk, fluks, permeat retentat atau konsentrat.*

INTRODUCTION

Kombucha tea had been known in various kind of tea, kind of Kombucha starter and preparation process. Composition of Kombucha tea, especially polyphenol content, makes into this drink as functional drink for anti oxidant, anti diabetes and anti cholesterol. Beside dominant polyphenol compound, such as L-theanine as anti stress compound, Kombucha tea with different composition gives added value on functional property. L-theanine is an unique amino acid analog of glutamine in green tea, which has an ability to stimulate ά wave production in human brain (0.5–3 Hz), increase dopamine and serotonin concentration and own important role in formation of neurotransmiter gamma-aminobutyric acid (GABA) followed by giving relaxation situation without cause of pulsate sensation (Adham, M.A., *et al*., 2006). To get L-theanine-rich green tea, separation and purification of green tea through membrane system (MF followed Nanofiltration, NF) under optimal process condition will increase L-theanine content larger than 100% when compared with L-theanine content in fresh tea leaves (1.5 – 3%) or approximately 50–70% from total free amino acids in tea leaves (Susilowati, *et al* ., 2009).

L-theanine has physical properties, such as savory taste, molecular weight (MW) of 174, soluble in water, melting point of 217 – 218 ºC, optical density (OD) + 7,0 and white crystal (Fu, D., 2006). Content of L-theanine is not only influenced by kind and variety of green tea, but also by treatment system of green tea. Local green tea from *C. assamica* with *Dewata* and *Pekoe* varieties and *C. sinensis* with *Arraca kiara* and *Arraca yabukita* varieties are always used for drink. Use of the tea kind in extraction, separation and purification of L-theanine are aimed to get functional compound to increase quality and economic value (Susilowati, *et al.*, 2009).

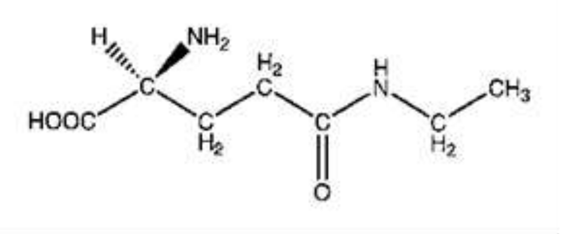


FIGURE 1
Chemical structure of L- theanine [L-Glutamic acid γ-(ethylamide) or Nγ-Ethyl-L-Glutamine].

Kombucha fermentation on green tea extract produced from MF membrane enables to get higher L-theanine content. This matter is caused by more much L-theanine (MW of 174 Dalton, Da.) passing in permeate (extract) when compared with it on the top membrane surface as retentate or concentrate due to smaller particle size (0.001 – 0.01 µm) (Anonymous, 2005) than pores size of MF membrane (0.2 µm) used (Susilowati, *et al.*, 2009). This substrate will facilitate Kombucha culture to act fermentation in order to get higher concentration of bioactive compound. Use of Kombucha culture as mixture of fungi and bacteria (acetic acid bacteria) generates Kombucha tea with different composition, particularly L-theanine. Kombucha culture contains acetate acid bacteria, such as *Acetobacter xylinum* and yeast (*Saccharomyces cereviseae*, *Saccharomyces ludwigii*, *Saccharomyces bisporus*, *Zygosaccharomyces* sp.) and several kinds of yeast (*Torolupsis* sp.) (Malbasa, *et.al.*, 2008). A symbiosis of bacteria and various fungi will produce lactic acid, vitamins, amino acids, antibiotic and other components (Jayabalan, *et. al.*, 2008). and produce specific taste drink of Kombucha tea like fresh fruit aroma or acids or vinegar (Dufresne & Farnworth., 2000) through assimilative and dissimilative chemistry reactions for 7 - 14 days of fermentation.

Separation and purification of fermentation product are needed to get concentrate or extract of Kombucha tea with specific composition in order to increase functional value. Selection on separation and purification using microfiltration (MF) membrane system are caused by ability of MF membrane to sieve macromolecular larger than 500.000 g/mol or particle size of 0.1 - 10 µm so that tea compound with particle size smaller than 0.2 µm will pass freely as permeate, such as caffeine, anthocyanine pigment (0.1 – 10 µm), polyphenol, organic acids, amino acids, vitamins and minerals (0.001 – 0.1 µm) (Anonymous, 2005). Purification of Kombucha tea produced through fermentation of green tea from various kinds and variety by means of stirred MF cell (SMFC) in laboratory scale (200 mL) enables it to result concentrate with higher bioactive compounds, particularly L-theanine. The concentrate of Kombucha tea can be used as Kombucha inoculum, functional drink or raw material of medicine for anti stress.

The objective of this experiment was to know process condition optimization and MF membrane performance in separating and purifying Kombucha tea with different kind of Kombucha tea and stirrer rotation speed so that it is produced extract (permeate) or retentate (concentrate) with the best composition of bioactive compound, especially L-theanine.

MATERIALS AND METHODS

Materials used in this experimental work were green tea from *Camellia sisnensis* varieties with *Arraca kiara and Arraca yabukita* grades and *Camellia* *assamica* varieties with *Dewata* and *Pekoe* grades purchased from a local manufacture (P.T. Chakra, Gambung, West Java, Indonesia), extract of local green tea produced by MF membrane at pump motor frequency of 20 Hz and pressure of 4 bar for 120 minutes, Kombucha colony, analytical reagent grade chemicals, and Commercial Polysulphone Microfiltration (MF) membrane (pore size of 0.2 µm, effective membrane area of 0.036 m^2, GRM-0.2-PP, Danish Separation Systems, Denmark).

Equipments used in this activity were Stirred MF Cell (SMFC) with capacity of 200 mL (Amicon-8200, U.S.A.), fermentation system in laboratory scale, incubator and laminar flow chamber, Spectrophotometer UV-1201, and glass ware.

Experimental design

Separation and purification of Kombucha tea were carried out with variation kinds of Kombucha tea from green tea extract with *Arraca kiara*, *Arraca yabukita*, *Dewata* and *Pekoe* grades fermented by Kombucha culture. Green tea extract purified by means of MF Module at operation pressure of 4 bar for 120 minutes was introduced as feed into SMFC. Kombucha tea was separated and purified via SMFC in laboratory scale (capacity of 200 mL) at stirrer rotation speed of 200 and 400 rpm, room temperature, and pressure of 30 psia for 30 minutes. Analysis were employed on feed, and permeate and retentate (as a result of MFM) covering Total Solids (Gravimetric method), Reducing Sugar (Somogyi-Nelson method), Total Acids (Titration method) (A.O.A.C., 1980), L-Theanine (Nynhidrin method) (Xiao, 2006) and Total Polyphenol (Folin-Denis Method) (Liu, S., 2006a). Process and analysis were conducted in duplicate. Data were processed in description based on result of average analysis.

Process steps

To separate, purify and yield Kombucha tea as healthy drink covered subsequently steeping of dry green tea leaves by adding hot water, separation and purification of steeped green tea through MF membrane module, fermentation of green tea permeate by Kombucha culture (starter), sieving of fermentation product as Kombucha tea, separation and purification of Kombucha tea through SMFC in laboratory scale. Process schematic of preparation and purification of Kombucha tea from local green tea permeate (extract) purified through MFM by means of SMFC was shown in Figure 2.

Extraction and purification of green tea through MF modul

Green tea suspension (*Arraca kiara*, *Arraca yabukita*, *Dewata* and *Pekoe* grades) was steeped by adding 1 part of dry green tea to 15 parts of hot destilled water (± 90 ºC, 15 minutes) and sieved through a 200 mesh sieve equipped in High Separation Frequency. The green tea suspension is used as feed in separating and purification of L-theanine through MF membrane of 0.2 µm as functional compounds (Susilowati, *et al.,* 2009). Feed fluid of green tea placed in feed tank of 9 L with overflow (drain) was subsequently pumped into pre-filtered in 200 µm, heating/cooling water system and

Lab Unit M20 module to result permeate and concentrate or retentate. The temperature of the recirculating feed solution was controlled and maintained constant at 23–24°C (room temperature) using a chiller. After process condition is stable, pump motor frequency was set to 20 Hz and operation pressure was set by regulating concentrate or retentate valve to 4 bars. During the MF process, both permeate and retentate or concentrate were recirculated to the feed tank in order to keep and maintain a steady operation condition and an effectively constant concentration (Anonymous, 2000).

Kombucha fermentation

Kombucha culture was inoculated by Kombucha starter in Potato Dextrose Agar (PDA) using ose needle and cultivated at 30 ^{o}C for 5 days. Kombucha starter liquid were prepared by pouring 100 mL of sterilized distilled water and shaked gentle to form microbe suspension, and separated. A quantity of 5% (v/v) of microbe suspension was poured into concentrate biomass of green tea, which had been added by sucrose (10%, w/v) and sterilized at 121^{o}C for 15 minutes. Fermentation was conducted in cheese cloth covered glass container under room temperature for 1 week. The whole fermentation activities were employed in laminar-flow chamber.

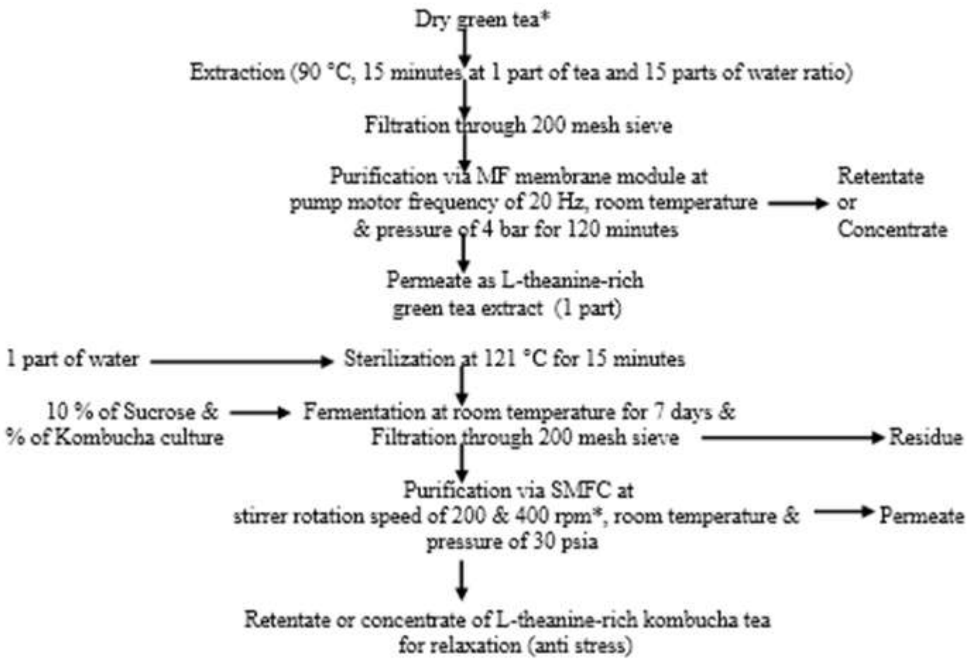
Purification of kombucha tea through stirred microfiltration cell (SMFC)

Separation and purification of Kombucha tea were conducted by means of SMFC. Figures 3a and 3b showed subsequent SMFC and Internal Arransement from SMFC. Before used, the cell containing a magnetic stirring bar and membrane was filled by 50 mL of distilled water to wet the membrane. Distilled water in the cell was then replaced with Kombucha tea with permeate of *Camellia sinensis* variety of *Arraca Kiara* grade, and operated at stirrer rotation speed of 200 rpm and pressure of 30 psia for about 30 minutes by providing nitrogen gas from cylinder until a more and less permeate steady flux had been achieved. Permeate passing from membrane was collected and recorded its volume to calculate permeate flux value, and retentate or concentrate in the cell was discharded. Both permeate and retentate or concentrate were then analysed. After the MF of Kombucha tea, the membrane was washed with distilled water. The MF was also the same except that Kombucha tea with another variety replaced Kombucha tea with *Camellia sinensis* variety *Arraca kiara* grade according to research design (Anonymous, 2002).

RESULTS

Characteristics of Local Green Tea Extract and Kombucha Tea

Local extract of green tea from Camellia sinensis variety with Arraca kiara and Arraca yabukita grades and Camellia assamica variety with Dewata and Pekoe grades purified through MF membrane module as enough clear suspension and brown color with gradation from clear brown to dark brown were subsequent shown in Figures 4a, 4b, 4c and 4d. Composition of green tea extracts from various kind and variety show a difference in



Legend : *research variables.

FIGURE 2

Process schematic of preparation and purification of Kombucha tea from local green tea permeate (extract) purified through MF membrane module by SMFC as functional drink for relaxation (anti stress).

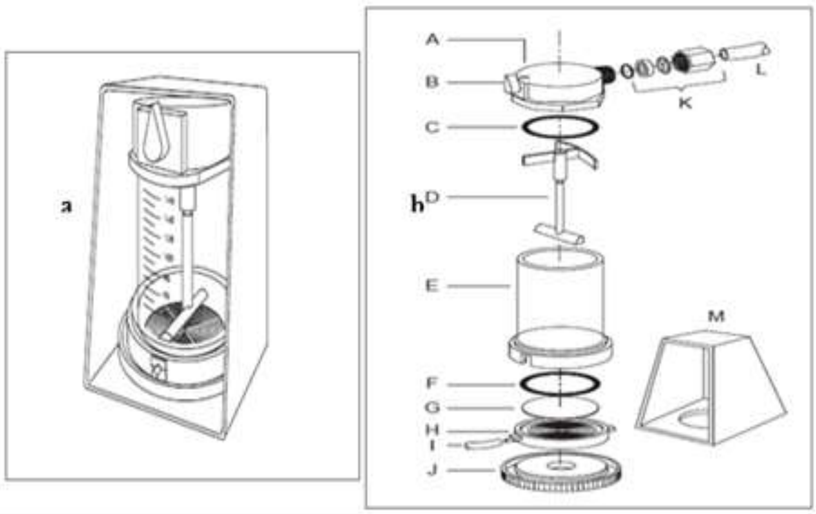


FIGURE 3

Schematic Representation of SMFC (a) and Internal Arransement from SMFC (b), where A, Cap Assembly; B, Pressure Relief Valve; C, O-Ring; D, Stirrer Assembly; E, Body; F, O-ring; G, MF Membrane of 0.2 µm; H, Membrane Holder; I, Elastomeric Tubing; J, Base; K, Tube Fitting Assembly; L, Tubing or plastic; and M, Stand Assembly [Anonymous, 2002].

product caused by initial composition of dry tea leaves and kind or variety. Treatment process of tea leaves affects also on composition and suspension color, in which green tea of Dewata and Pekoe grades were prepared fan firing process (direct heating at temperature larger than 140 ºC), while Arraca kiara and Arraca yabukita grades were produced by steaming process. Through steeping process at 1 part of tea leaves and 15 part of hot water at 90 ºC for 15 minutes was produced green tea water steeping followed by sieving and MF membrane module under pump motor frequency of 20 Hz, room temperature and pressure of 4 bar for 120 minutes would generate part passing via MF membrane as permeate and part retained on the top membrane surface as retentate or concentrate.

Use of MF membrane with pore size of 0.2 µm enables it to pass freely more much L-theanine in permeate than that in retentate or concentrate because its particle size (MW of 174 Da. or 0.001 – 0.01 µm in range) was smaller than MF membrane pore size (0.2 µm). Dominant components in green tea (polyphenol) have various concentrations for 4 kinds of green tea, in which green tea of Arraca Yabukita grade has the highest component concentration when compared other kinds of green tea. Total solids concentration in extract or permeate of green tea of Arraca yabukita grade gives similar content. L-theanine and total polyphenols from 4 kinds of green tea vary, but from the whole green tea kinds, L-theanine content is higher when compared by L-theanine content in fresh tea leaves (1 – 1.5 %). Whereas, total polyphenols does not indicate significant difference, but the concentration range is lower than in retentate or concentrate (Susilowati, et al., 2009).

Table 1 show subsequent green tea composition in extract or permeate of tea (Camellia sp.) leaves as raw material in fermentation of Kombucha tea.

Initial composition of material after fermentation process using Kombucha culture showed difference in organoleptic aspect (color, viscosity, taste, aroma) due to its occurence of composition change. Kombucha tea seems thicker, fresh aroma like vinegar aroma or organic acids, sour, less sweet and darker. Taste and fresh aroma like alcohol is caused by its occurence of organic acids expressed as total acid. More dense fungi spore is formed at the top suspension surface. Figures 5a, 5b, 5c and 5d showed subsequently biomass of Kombucha tea from green tea extract of Arraca kiara, Arraca yabukita, Dewata and Pekoe grades as a result of fermentation by Kombucha culture (5%) at room temperature for 7 days.

Table 2 showed composition of Kombucha tea after fermentation. Diluted green tea extract and water with ratio 1:1 was used in fermentation, therefore its composition becomes lower than initial composition of material (extract or permeate as a result of MFM), particularly L-theanine and total polyphenol. From result of this fermentation, total solids become higher as a consequency of distribution of Kombucha culture, sucrose and metabolite product during fermentation. Other possibilities are a part of components which is used to grow Kombucha tea, so that it is occurred concentration deficit, especially L-theanine as source of protein for microbe nutrition.

TABLE 1
Composition of extract (permeate) of local green tea

Grade of greentea extract*	Components		
	L-Theanine (%, w/w)	Total Polyphenol (%, w/w)	Total Solids (%, w/w)
Arraca Kiara.	13.395	9.481	1.685
Arraca Yabukita.	9.435	16.187	1.946
Pekoe	11.972	13.486	1.658
Dewata.	10.721	10.721	1.343

FIGURE 4
Extract (permeate) of green tea from Camellia sinensis variety with Arraca kiara (a) and Arraca yabukita grades (b), and Camellia assamica variety with Dewata (c) and Pekoe (d) grades.

FIGURE 5
Biomass of Kombucha tea from green tea extract of Arraca kiara (a), Arraca yabukita (b), Dewata (c) and Pekoe grades (d) as a result of fermentation by 5% (v/v) of Kombucha culture and 10% (w/v) of sucrose at room temperature and 7 days of fermentation.

TABLE 2
Composition of Kombucha tea as feed in purification via SMFC using MF membrane of 0.2 µm.

Green tea grade as feed	Components				
	L-Theanine (%, w/w)	Total Polyphenol (%, w/w)	Reducing Sugar (mg/mL)	Total Solids (%, w/w)	Total Acidsas acetic acid (%, v/v)
Arraca Kiara.	2.4534	17.1376	51.50	4.0450	0.5270
Arraca Yabukita.	2.6524	5.0556	59.50	12.5046	0.3073
Pekoe	4.1292	8.7600	31.75	7.7261	0.8122
Dewata.	1.6668	14.4667	27.25	7.3469	1.7564

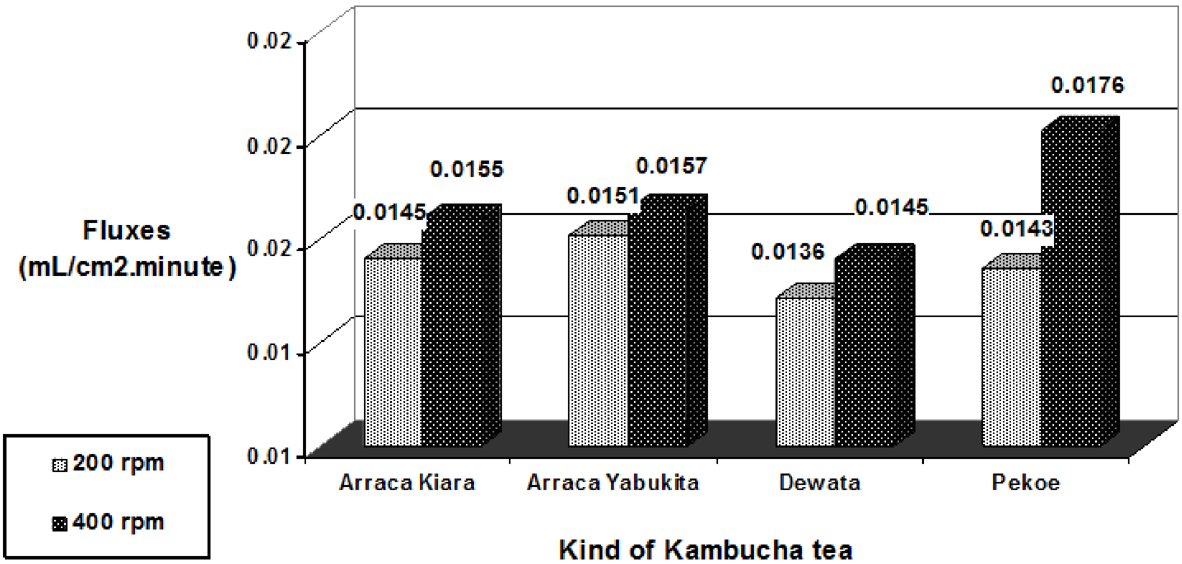


FIGURE 6
Relationship between types of Kombucha tea and permeate flux value as a result of purification by SMFC at stirrer rotation speed of 200 rpm and 400 rpm and pressure of 30 psia.

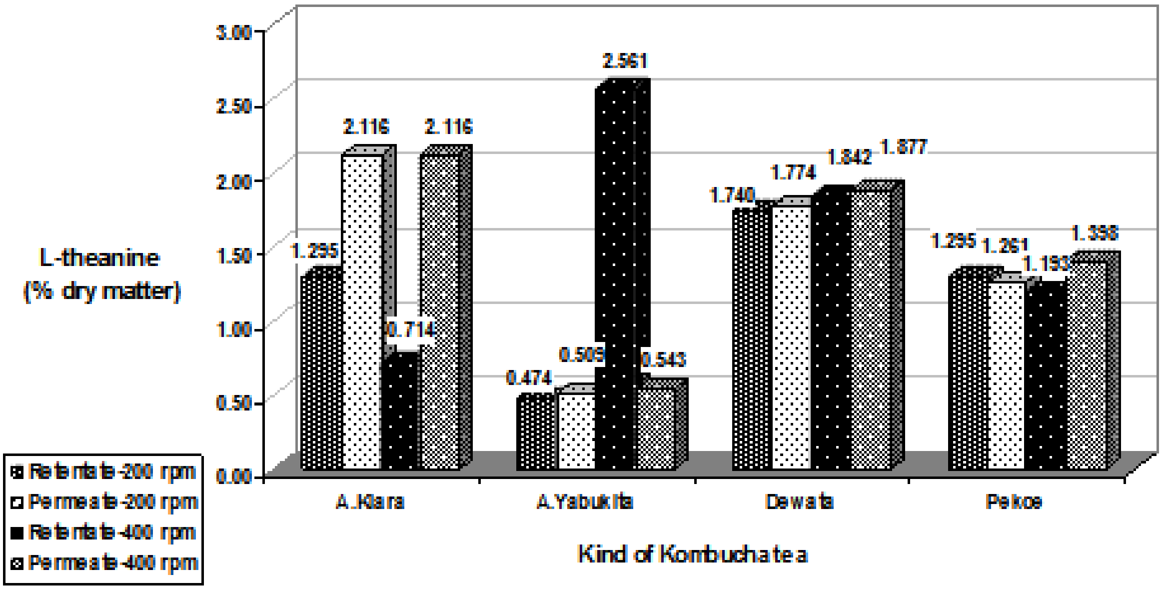


FIGURE 7
Relationship between types of Kombucha tea and L-theanine content in retentate and permeate from *Arraca kiara*, *Arraca yabukita*, *Dewata* and *Pekoe* grades as a result of purification by SMFC at stirrer rotation speed of 200 rpm and 400 rpm and pressure of 30 psia.

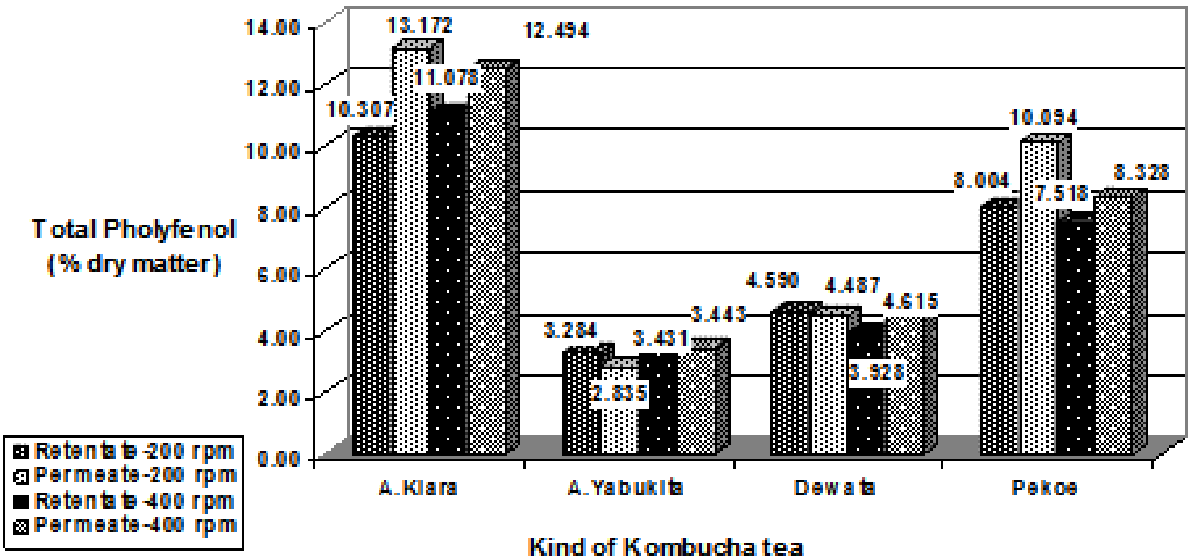


FIGURE 8
Relationship between types of Kombucha tea and total polyphenol content in retentate and permeate from *Arraca kiara*, *Arraca yabukita*, *Dewata* and *Pekoe* grades as a result of purification by SMFC at stirrer rotation speed of 200 rpm and 400 rpm and pressure of 30 psia.

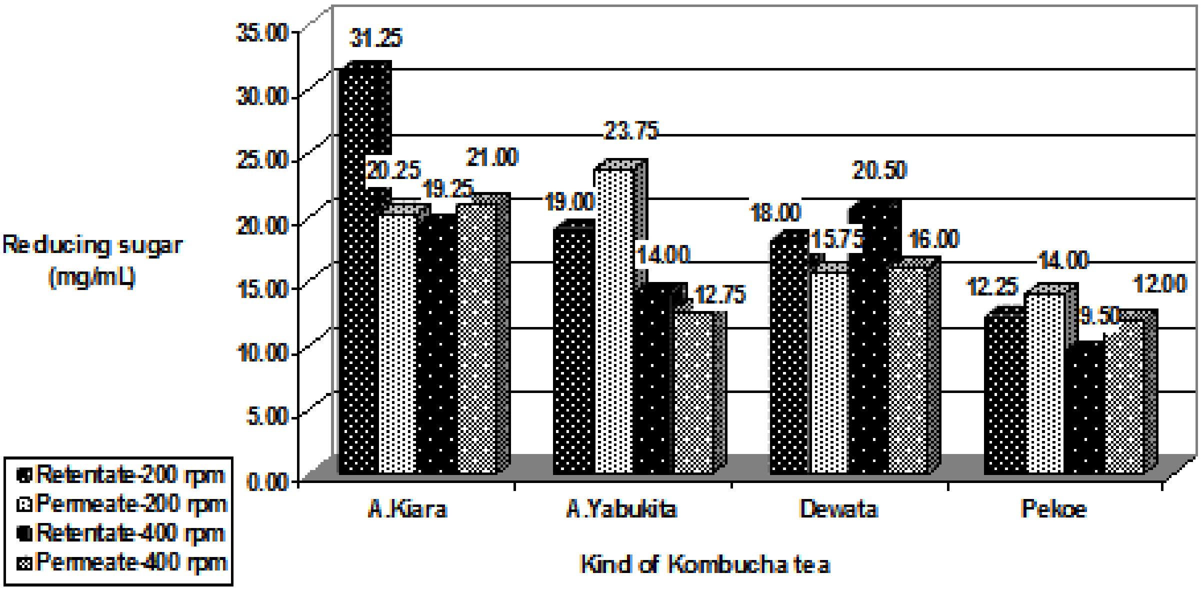


FIGURE 9
Relationship between types of Kombucha tea and reducing sugar content in retentate and permeate from *Arraca kiara, Arraca yabukita, Dewata* and *Pekoe* grades as a result of purification by SMFC at stirrer rotation speed of 200 rpm and 400 rpm and pressure of 30 psia.

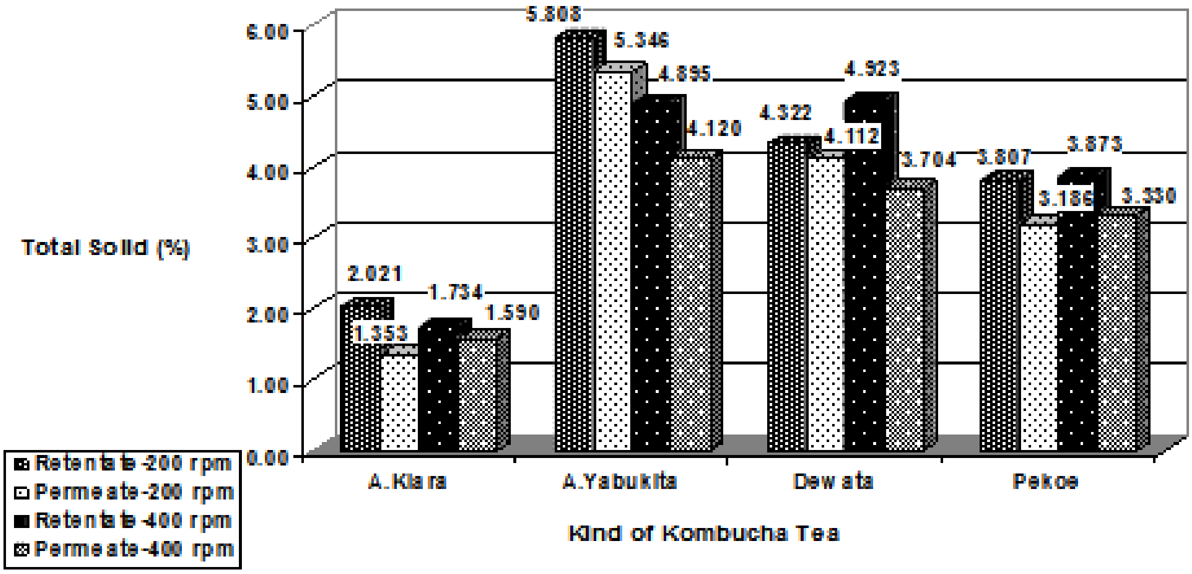


FIGURE 10
Relationship between types of Kombucha tea and total solids content in retentate or concentrate and permeate from *Arraca kiara, Arraca yabukita, Dewata* and *Pekoe* grades as a result of purification by SMFC at stirrer rotation speed of 200 rpm and 400 rpm and pressure of 30 psia.

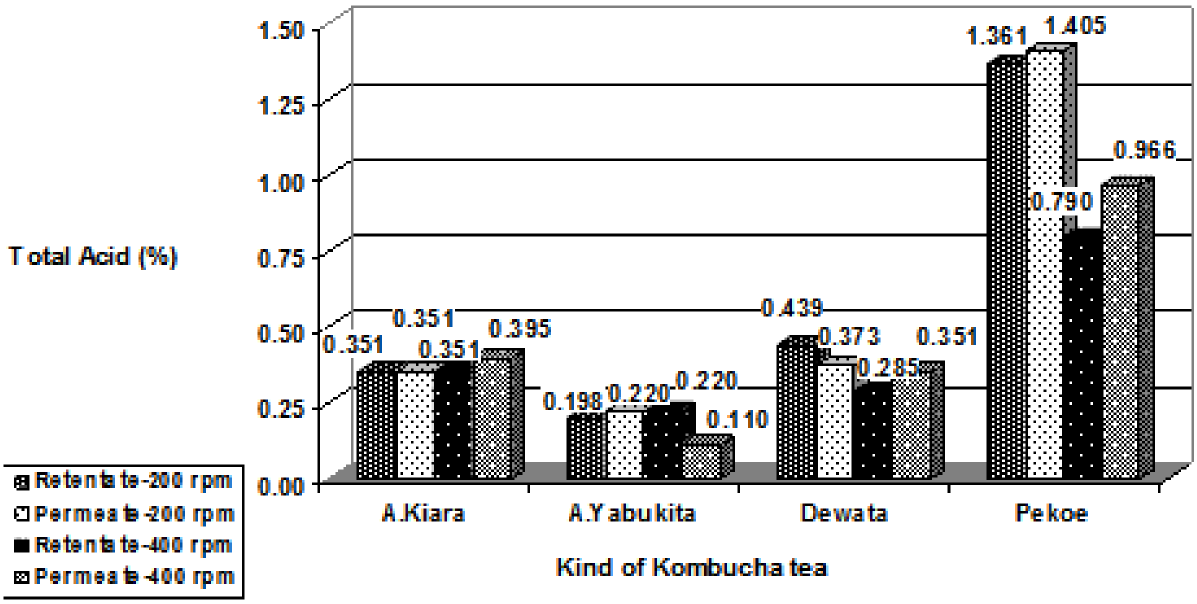


FIGURE 11
Relationship between types of Kombucha tea and total of titratable acids content in retentate and permeate from *Arraca kiara*, *Arraca yabukita*, *Dewata* and *Pekoe* grades as a result of purification by SMFC at stirrer rotation speed of 200 rpm and 400 rpm and pressure of 30 psia.

Difference in the permeate flux value was caused by operation condition factors (stirrer rotation speed and pressure) and total solids concentration. With higher stirrer rotation speed at fixed pressure (30 psia) gave higher driving force, so that flow rate of feed and permeate flux value became faster because tea particles would be focused on the top membrane surface. Initial total solids concentration for Kombucha tea kind with *Arraca kiara*, *Arraca yabukita*, *Dewata* and *Pekoe* grades *were* subsequently 4.045, 12.5046, 7.7261 and 7.3469%. This matter was possibility caused by enough high polyphenol content (14.4667%) retained on the top membrane surface, so that it increases permeate flux value for Kombucha tea kind with *Pekoe* grade when compared with other Kombucha tea. Polyphenol or catechins were dominan components in green tea with MW range of 200-600 Da. and ranged in 25–35% (dry weight) from the whole components of green tea (Liu, 2006b).

L-theanine (%, dry weight)

SMFC system using MF membrane of 0.2 µm showed that process treatment of Kombucha tea at stirrer rotation speed of 200 and 400 rpm, and pressure of 30 psia for 30 minutes were able to affect on L-theanine optimization both in permeate and in retentate or concentrate, as shown in Figure 7. At stirrer rotation speed of 200 rpm, SMFC on Kombucha tea kind with *Arraca kiara*, *Arraca yabukita*, *Dewata* and *Pekoe* grades retain L-theanine in retentate 1.295, 0.474, 1.74 and 1.295% (dry weight) and pass freely in permeate 2.116, 0.508, 1.774 and 1.261%. This condition showed that SMFC system of Kombucha tea kind with *Arraca kiara* grade was able to pass freely optimum L-theanine in permeate (86.25%) from prior to process (2.4534%, dry weight) to after process (2.116%, dry weight). Different trend seems at stirrer rotation speed of 400 rpm, in which SMFC on Kombucha tea kind with *Arraca kiara, Arraca yabukita, Dewata* and *Pekoe* grades retain subsequent L-theanine in retentate or concentrate 0.714, 2.561, 1.842 and 1.193% (dry weight) and pass freely in permeate 2.116, 0.543, 1.877 and 1.398%. Under this condition, optimization value was reached by Kombucha tea kind of *Arraca yabukita* grade with L-theanine in retentate or concentrate 95.55% from before process (2.6524%, dry weight) to after process (2.561%, dry weight). Differences in L-theanine content in retentate and permeate were possibility caused by interaction between process condition and particle size of L-theanine. At higher rotation speed of stirrer (400 rpm) enables larger driving force of particles on membrane surface supported by operation process (30 psia). In this matter, L-theanine in retentate or concentrate was more much than in permeate, in spite of low MW of L-theanine (174 Da.) and particle size range of 0.001 – 0.01 µm (Anonymous, 2005) or smaller when compared to pores size of MF membrane (0.2 µm).

This matter is caused by its occurence of *fouling,* its accumulation of solutes caused by interaction of inter components both physical and chemical to form *cake* combatting solute to pass freely (Cheryan, 1998).

This phenomenon is indicated for *Arraca yabukita* and *Pekoe* grades, but for *Arraca kiara* and *Dewata* grades, in which L-theanine passes more much in permeate. This trend seems also under stirrer rotation speed of 200 rpm, in which L-theanine in permeate is more much than retentate or concentrate (*Arraca kiara, Arraca yabukita* and *Dewata* grades). With stirrer rotation speed of 200 rpm, L-theanine is possibility able to pass freely, so that *cake* does not formed. It had been demonstrated that system of SMFC acts as a *dead end* system, in which direction of filtration flow forms 0^o or 90^o angle on the top membrane surface, so that components rejected or retained in fluid become more and more high during process (Keith Scott, 1998). In the whole processes of SMFC, optimum condition was reached at stirrer rotation speed of 400 rpm for *Arraca yabukita* grade and gave L-theanine concentration 2.5605% (dry weight) in retentate from the whole processes when compared to feed (2.6524%, dry weight) or MF efficiency larger than 96.53%.

Total polyphenol (%, dry weight)

Main chemical compound in tea leaves are polyphenol or catechins (25 – 35%, dry weight) consisted of complex cathechines, such as flavanols, flavanones, anthocyanin & leucoanthocyanidins, phenolic acids & depsides as an antioxidant, and can block its occurence of cancer growth (Liu, 2006b). Purification process of Kombucha tea from *Arraca kiara, Arraca yabukita, Dewata* and *Pekoe* grades by means of SMFC at stirrer rotation speed of 200 rpm were subsequent able to penetrate freely total polyphenol in permeate 13.172, 2.835, 4.487 and 10.094% and retained subsequent total polyphenol in retentate or concentrate 10.3067, 3.2838, 4.5896 and 8.0044 (%, dry weight). At this condition seemed that SMFC of Kombucha tea of *Arraca kiara* grade was able to penetrate optimum polyphenol (13.172%, dry weight) in permeate or lolos 76.86% from feed (17.1376%, dry weight).

Different trend seems at process treatment with stirrer rotation speed of 400 rpm, in which SMFC system of Kombucha tea of *Arraca kiara* grade was subsequent able to cross freely total polyphenol in permeate 12.494, 3.443, 4.615 and 8.328% and retained subsequent total polyphenol in retentate or concentrate 11.078, 3.431, 3.928 and 7.518 (%, dry weight). Based on total polyphenol, optimum condition was reached by using Kombucha tea of *Arraca kiara* grade and crossing freely total polyphenol in permeate 12.4994% or passing efficiency of 72.905% from feed (17.1376%, dry weight).

From the whole processes, Kombucha tea of *Arraca kiara* grade gave the highest total polyphenol both in retentate or concentrate and in permeate under stirrer rotation speed of 200 and 400 rpm, as shown in Figure 8. When compared to other components in Kombucha tea, polyphenol is a sufficient dominant component. In fermentation of steep of local green tea (1 part of green tea : 15 parts of water) using culture Kombucha was occurred a decrease of total polyphenol due to its degradation in formation of other organic acids (Susilowati, *et al.,* 2010). Particle size of polyphenol was 0.001–0.01µm (Anonymous, 2005) and MW of 200 – 600 kDa., so that it would pass more much in permeate because particle size of polyphenol was smaller than pores size of MF membrane (0.2 µm), nevertheless total polyphenol could also retained in retentate or concentrate when it is happened *fouling.* This matter seemed in retentate from Kombucha tea of *Arraca yabukita* and *Dewata* grades retaining polyphenol 3.284 and 4.59% under stirrer rotation speed of 200 rpm. This is possibility occurred on total polyphenol with high MW. It had been indicated that tea polyphenols, such as (+)-Catechin (C), (-)-Epicatechin (EC), (-)-Gallocatechin (GC), (-)-Epigallocatechin (EGC), (-)-Epicatechin gallate (ECG), (-)-Catechin gallate (CG), (-)-Epigallocatechin gallate (EGCG), (-)-Gallocatechin gallate (GCG), (-)-Epicatechin digallate (ECDG), and (-)- Epigallocatechin digallate (EGCDG) (Liu, 2006b) have various MW so that higher polyphenol compound was enabled to be retained on the top membrane surface due to lower rotation speed of stirrer (200 rpm).

Reducing sugar (mg/mL)

Reducing sugar in Kombucha tea is glucose residue after fermentation process, in which glucose is source of carbon in producing ethanol, acetaldehyde, organic acids and is affected by total starter, environmental condition and invertase enzyme activity in formatting metabolite.

After fermentation process, sugar was hydrolyzed to reducing sugars (glucose and fructose) On the other hand, sucrose is also used by *A. xylinum* bacteria to form cellulose as filament and floats it on the media surface (Anonymous, 2008). Purification process of Kombucha tea from *Arraca kiara, Arraca yabukita, Dewata* and *Pekoe* grades through SMFC at stirrer rotation speed was subsequent able to cross freely reducing sugar 20.25, 23.75, 15.75

and 14 mg/mL and retained subsequent reducing sugar in retentate or concentrate 31.25, 19, 18 and 12.25 mg/mL or optimum (31.25 mg/mL) in retentate of Kombucha tea from *Arraca yabukita* grade and was retained 52.52% from feed (59.5 mg/mL). Different trend seemed at process treatment with stirrer rotation speed of 400 rpm, in which SMFC system was able to pass freely reducing sugar in permeate 21, 12.75, 16, and 12 mg/mL, respectively and retained reducing sugar in retentate or concentrate 19.25, 14, 20.5 and 9.5 mg/mL or optimum condition was reached for *Arraca kiara* grade in permeate 21 mg/mL and pass 40.78 % from feed (51.5%, dry weight), as shown in Figure 9.

Reducing sugar is one of the important indicators of the Kombucha culture growth, in which sucrose is source of carbon used to metabolism in producing bioactive compounds in green tea. Sugar particle with size of 0.0008 – 0.001 µm (200 – 400 Da.), which is smaller than pore size of MF membrane (0.2 µm) (Anonymous, 2005; Woemer, 2004) should pass more much in permeate except its presence of *fouling*. Another possibility is its occurence of degrading to smaller molecules unit passing freely as permeate. Reducing sugar is sugar molecule (monosaccharides or disaccharide) having reducing property because hydroxyl bond (OH) is reactive (Belitz and Grosch, 1999) with high solubility in water.

Total Solids (%)

Total solids are accumulation of all components (soluble and insoluble) of Kombucha tea. Purification process of Kombucha tea from *Arraca kiara*, *Arraca yabukita*, *Dewata* and *Pekoe* grades through SMFC at stirrer rotation speed of 200 and 400 rpm, and pressure of 30 psia gave successfully separation because total solids in retentate was more much than in permeate, as shown in Figure 10. Purification process of Kombucha tea from *Arraca kiara*, *Arraca yabukita*, *Dewata* and *Pekoe* grades through SMFC at stirrer rotation speed of 200 rpm was subsequent able to pass freely total solids in permeate 1.353, 5.346, 4.112 and 3.186%, and retained total solids in retentate or concentrate 2.021, 5.808, 4.322 and 3.807%, respectively. While, purification process of Kombucha tea from *Arraca yabukita* grade was able to reject and retain optimum total solids (5.808%) in retentate 46.45% when compared to feed (12.505%). Different trend seemed at process treatment under stirrer rotation speed of 400 rpm, in which SMFC system was able to pass total solids in permeate on the similar Kombucha tea 1.59, 4.12, 3.704, and 3.33%, respectively, and rejected or retained total solids in retentate or concentrate 1.734, 4.895, 4.923 and 3.873%, respectively. In this condition, optimum total solid was reached for *Dewata* grade and retained total solid in retentate 4.923% or passed 63.72 % from feed (7.7261%).

For the whole processes seemed that optimization of Kombucha tea purification on total solids is not affected by stirrer rotation speed, but it is affected by kind of Kombucha tea. This matter was indicated by Kombucha tea from *Arraca kiara* and *Arraca yabukita* grades, which was able to retain more much total solids in retentate or concentrate 2.021 and 5.808 % at stirrer rotation speed of 200 rpm than that stirrer rotation speed of 400 rpm, 1.734 and

4.895 %. With higher speed of stirrer rotation (400 rpm), more much retained total solids in retentate or concentrate due to driving force on SMFC system.

This matter was displayed by Kombucha tea from *Dewata* and *Pekoe* grades, which was able to reject and retain more much total solids in retentate or concentrate at stirrer rotation speed of 400 rpm, 4.923 and 3.873% when compared with stirrer rotation speed of 200 rpm, 4.322% and 3.807%. Difference in total solids from the result of SMFC system was possibility caused by difference in initial composition from each component in Kombucha tea, its occurence of fouling and deficite of water mass to penetrate membrane pores, so that it compact retained material component on the top membrane surface. Kombucha tea is a biomass, which contains still culture Kombucha, a mixture of fungi and acetic acid bacteria to contribute total solids.

Total of titratable acids (%)

Total acids indicated the ability of Kombucha culture to ferment and to metabolite substrate (sugar) as source of carbon in order to microbes (yeast and acetic acid bacteria) (Malbasa, *et al.*, 2008). Total acids as organic acids (acetic acid, acetaldehyde) are produced through fungi hydrolysis on sucrose to form glucose and fructose, and converted to gluconic acid and acetic acid. The organic acids have important role on flavor, taste, and aroma of Kombucha tea, in which they have particle size in range of 0.0001–0,001 μm (Anonymous, 2005; Woemer, 2004), so that they are enabled to pass in permeate. Under stirrer rotation speed of 200 rpm, SMFC system was able to pass subsequently freely total acids in permeate of Kombucha tea from *Arraca kiara, Arraca yabukita, Dewata* and *Pekoe* grades of 0.351, 0.22, 0.373 and 1.361%, and retained total acids in retentate or concentrate 0.3512, 0.198, 0.439 and 1.405%, respectively as demonstrated in Figure 11. In other words, total acids of Kombucha tea from *Pekoe* grade in retentate or concentrate was reached in the highest concentration when compared to total acids for the whole treatment processes. In this condition seemed that SMFC system was able to retain optimum total acids (1.405%) in retentate or concentrate of Kombucha tea from *Pekoe* grade or total acids were retained 79.99% from prior to process or feed (1.7564%). Almost similar trend seemed also at stirrer rotation speed of 400 rpm, in which total acids in permeate were subsequent reached 0.351, 0.22, 0.285 and 0.79% and were rejected and retained in retentate or concentrate 0.351, 0.11, 0.351 and 0.966%, respectively. At this condition, optimum total acids were reached by Kombucha tea from *Pekoe* grade, that was able to pass total acids in permeate 0.966% or 55% from before process or feed (1.7564%). Based on total acid optimum in permeate and retentate or concentrate Kombucha tea from *Pekoe* grade was kind of tea with the best purification level, in which SMFC system with stirrer rotation speed of 200 rpm was able to pass the highest total acids in permeate (1.361%) and to retain higher total acids in retentate or concentrate (1.405%) when compared to other treatment processes.

CONCLUSIONS

Stirred MF Cell (SMFC) using MF membrane of 0.2 µm has potential use in purification of Kombucha tea from local extract of green tea. Separation and purification of each component in Kombucha tea was affected by process condition, and component and specific properties for every Kombucha tea kind. Optimization for each component yielded specific permeate and retentate or concentrate for each Kombucha tea kind. Stirrer rotation speed of 400 rpm resulted better composition than 200 rpm. Based the highest L-theanine concentration, SMFC at stirrer rotation speed of 400 rpm was subsequently able to retain 29.1% of L-theanine component of Kombucha tea with *Arraca kiara*, 96.53% of *Arraca yabukita*, 44.62% of *Dewata*, and 71.54% of *Pekoe* grades in retentate or concentrate when compared with L-theanine component of Kombucha tea prior to process. In other words, optimization condition was reached by retentate or concentrate of *Arraca yabukita* grade with L-theanine of 2.5605%, total polyphenol of 3.4314%, reducing sugar of 14 mg/mL, total solids of 4.8947% and total of titratable acids of 0.2195%, respectively.

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