CONCENTRATION OF DIAFILTRATE IN LOCAL GREEN TEA (*Camellia sinensis*) WITH *Arraca yabukita* GRADE THROUGH NANOFILTRATION MEMBRANE AS ANTI STRESS COMPOUND

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ABSTRAK

Proses pemekatan diafiltrat teh hijau lokal (*Camellia sinensis*) grade *Arraca yabukita* merupakan rangkaian multifiltrasi untuk memisahkan dan memekatkan L-theanine dari komponen teh hijau lainnya yang didominasi oleh polyfenol. L-theanine merupakan komponen yang berperan sebagai senyawa untuk relaksasi dan berpotensi untuk anti stres. Pemekatan dilakukan dengan menggunakan modul nanofiltrasi (NF) pada tekanan proses 25 bar, kecepatan motor pompa 25 Hz, selama 0, 30, 60, 90, 120 dan 150 menit, dan pada suhu ruang (~ 23 - 25°C). Hasil penelitian menunjukkan bahwa sistem NF memisahkan L-theanine dengan sempurna dimana L-theanine lebih banyak tertahan pada retentat/konsentrat dari pada lolos dalam permeat. Selektifitas membran nanofiltrasi terhadap L-theanine, protein terlarut, total polyfenol dan total padatan tercapai masing-masing sebesar 97,92 %, 99,75 %, 10,38 % dan 99,78 %. Semakin lama proses NF akan meningkatkan L-theanine, total polyfenol, protein terlarut dan total padatan dalam retentat, namun menurunkan nilai fluks permeat. Konsentrasi L-theanine tertinggi dicapai pada waktu pemekatan optimal 150 menit. Pada kondisi ini menghasilkan nilai fluks sebesar 12,22 Liter/m^2.jam dengan kandungan L-theanine sebesar 7,2117 %, total polyfenol 9,84 %, protein terlarut 2 mg/mL dan total padatan 1,2102 %. Permeat berpotensi sebagai minuman fungsional untuk relaksasi.

Kata kunci: diafiltrat, L-theanine, teh hijau (*Camellia sinensis*), konsentrat, Nanofiltrasi (NF)

ABSTRACT

Diafiltrate concentration process of local green tea (*Camellia sinensis*) of *Arraca yabukita* grade is a multi filtration series to separate and concentrate L-theanine from other green tea components dominated by polyphenol component. L-theanine is a component having important role as anti stress compound for relaxation activity. Concentration was performed by using nanofiltration (NF) membrane module at pump motor frequency of 25 Hz, room temperature (~ 23 - 25°C) and operation pressure of 25 bar for 0, 30, 60, 90, 120, and 150 minutes. The experiment result showed that NF membrane system was technically able to separate successfully L-theanine, in which L-theanine was retained more much in concentrate (retentate) than in permeate. Selectivity of NF membrane on L-theanine, dissolved protein, total polyphenol, and total solids components were reached at 97.92 %, 99.75 %, 10.38 %, and 99.78 %, respectively. The long time of NF process would drop permeate flux value, and increased L-theanine, dissolved protein, total polyphenol, and total solids in retentate. Based on the highest L-theanine content, optimal concentration time was reached in 150 minutes. This condition yielded permeate flux value of 12.22 L/m^2.hour, and showed contents of L-theanine of 7.211 %, dissolved protein of 2 mg/mL, total polyphenol of 9.84 %, and total solids of 1.2102 %. Permeate has a potential utilize as a functional drink for relaxation activity.

Keywords: diafiltrate, L-theanine, green tea (*Camellia sinensis*), concentrate, Nanofiltration (NF)

INTRODUCTION

L-theanine [Nγ-Ethyl-L-glutamine or L-Glutamic acid γ-(ethylamide)] is an unique amino acid analog of glutamine, found in green tea (leave, branch and stem) and *Cunnighamella echinulata* fungus (Jiayou Li *et al.*, 2006) as anti stress compound. L-theanine have physical properties, such as savory taste, molecular weight (MW) of 174, soluble in water, melting point of 217 – 218 °C, optical density $[\alpha]_d$ 20 + 7.0 and white crystal. While, chemical properties of L-theanine are isoelectric point of pH 5.7 and reaction with ninhydrin will determine its quality (Fu, 2006). Main ability of L-theanine is to stimulate a wave of ά in brain (0.5 – 3 Hz), increase dopamine and serotonin in order to give relax condition (Liu, Z., 2006a) and form neurotransmiter gamma-aminobutyric acid (GABA) to get suitable or relaxation situation (Xiao, W., 2006; Fu, D., 2006).

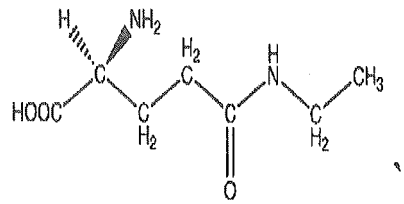

Figure 1. Chemical structure of L-theanine (Nγ-Ethyl-L-glutamine)

Green tea diafiltrate of *Arraca yabukita* grade is produced through a series of multi filtration process to separate L-theanine from components in green tea dominated by polyphenol component. Multi filtration process of green tea are subsequent green tea extraction to result green tea steep, green tea steep filtration through a 200 mesh sieve to get green tea filtrate as feed, purifying filtrate of green tea through microfiltration (MF) membrane of 0.15 μm to yield microfiltered permeate, concentration of microfiltered permeate using NF membrane to generate concentrate (retentate), and Diafiltration-Nanofiltration (DF-NF) hybrid process of NF concentrate (retentate) to separate L-theanine component from polyphenol component present in NF concentrate (retentate). In the DF-NF hybrid process, optimal process condition is reached at Number of Diafiltration (N_d) of 0.2 with polyphenol reduction of 14.89 % from polyphenol prior to DF-NF hybrid process (N_d of 0) (Susilowati, A *et al.*, 2009). To concentrate green tea diafiltrate of *Arraca yabukita* grade is performed via NF membrane module. Selection of NF module is caused by NF membrane with pores size ranging 1 – 10 nanometer (nm), which is able to separate components with Molecular Weight (MW) of 150 Dalton (Da.), such as monovalent anion, di- & multivalent anion, and organic compounds with MW of 300 Da. In its operation, to pass particles via pores in NF membrane is needed operation pressure of 7 – 30 bar (Eriksson, P., 1988). L-theanine (MW of 174) and polyphenol (10 – 12 components, MW 200 – 600) is rejected and retained on the top membrane surface as concentrate (retentate). Concentration or separation process of components in diafiltrate through NF membrane can be occurred by difference in properties amongst compounds separated,

such as difference in molecule size, interaction between dissolved compounds and membrane (solubility, diffusion) or another physical difference (molecule diameter). Difference in property amongst components separated become more and more wide, separation result will be occurred effectively and successfully. This property combination enables NF membrane to be effective in separation of organic solute mixture of low MW (neutral or charge) and salts (Van der Bruggen B *et al.,* 1999). Various components in green tea and application of NF membrane type with high rejection will affect on selectivity level of membrane in this separation, besides NF operation condition, such as difference in separated compound properties (size and molecule diameter), interaction amongst dissolved compounds with membrane (solubility, diffusion) and NF process condition (flow rate, temperature, pressure and time) (Raman, L.P *et al.*, 1994).

The aim of this experiment was to find out effect of concentration time of green tea diafiltrate of *Arraca Yabukita* grade through NF membrane on concentrate (retentate) and permeate compositions at pump motor frequency of 25 Hz, room temperature and operation pressure of 25 bar as functional compound for relaxation purpose.

METHODS

Main raw materials were local dry granular green tea of *Arraca yabukita* grade as a steam process result purchased from Tea Plantation of P.T. Kabepe Chakra, Gambung, Bandung (West Java, Indonesia), green tea diafiltrate as a multi filtration result using MF and NF membranes, NF Thin Film Composite membrane on Polyester (NF-99-PE) with total effective surface area of 0.036 m^2 (diameter 20 cm) (Danish Separation Systems, DSS, Denmark) (Anonymous, 2000), chemical reagents of analytical grade of ninhydrin, tin(II)chloride, di-sodium hydrogen phosphate, potassium dihydrogen phosphate, standard L-theanine, buffer and solution of phosphorus acid pH 8, and pure water purified by the reverse osmosis (RO) membrane.

Equipments utilized in this experiment were extraction unit in semi pilot scale (15 – 25 L), heater, High Separation Frequency equipped by 80 and 200 mesh sieve (Retsch, Germany), plate & frame type cross-flow membrane filtration modul (LabUnit M20, DSS, Denmark) equipped by high pressure pump of Positive Displacement Pump Rannie 25,38 (flow rate 3.5 – 15 L/minute and trans-membrane pressure of 50 bar) and glass ware.

The experiment was carried out by preparing granular green tea purchased from P. T. Kabepe Chakra, Gambung, Bandung (West Java, Indonesia). Separation and recovery L-theanine from green tea steep was subsequently carried out through extraction, filtration via a 80 mesh and 200 mesh sieves, removal of metal, and purification by means of 0.2 µm MF membrane module at pump motor frequency of 20 Hz (7 L/minute), room temperature and operation pressure of 4 bar for 120 minutes. Analysis was conducted on green tea extract before separation (feed) and after separation (permeate and concentrate/retentate) covering total solids (Gravimetric method), dissolved protein (Lowry) (A.O.A.C., 1980), total polyphenol (Folin-Denise method) (Liu, Z., 2006b) and L-theanine (Ninhydrin method) (Xiao, W., 2006).

Membrane filtration systems and methods

Three ways of membrane filtration systems were used for this experiment. One is a multi filtration (MF & NF) system as first concentration step, two is a discontinuous DF-NF hybrid process and the other is a concentration via NF membrane as second concentration step. Permeate flux value are measured in constant operation pressure, the tempearature effects were neglected.

NF concentrate (retentate) of green tea extract was produced through a series of process, such as adding 15 parts of hot water (± 90 °C) to 1 part of dry granular green tea, agitating for 5 minutes, allowing to steep for 15 minutes and filtering via a 200 mesh sieve to obtain filtrate and residue and purifying filtrate by MF membrane of 0.2 µm at pump motor frequency of 20 Hz (7 L/minutes) and operation pressure of 4 bar for 120 minutes to get permeate as green tea extract and concentrate (retentate). Green tea extract was then concentrated by means of NF membrane under pump motor frequency of 25 Hz (7.5 L/minute) and operation pressure of 25 bar for 120 minutes to result NF concentrate (retentate) and permeate. This NF concentrate (retentate) is then used as a feed in DF-NF hybrid process in order to separate L-theanine component. At the end of each run, the membranes were thoroughly flushed with RO water. The membranes were then cleaned in place using 4 % NaOH solution before storing in 1 % Sodium Azide till the subsequent run (Susilowati, A *et al.*, 2009).

Discontinuous DF-NF hybrid process aims to separate and wash L-theanine component from polyphenols present in green tea. Discontinuous DF-NF hybrid was performed in the batch mode, i.e., the permeate flux being compensated by an equal input of RO water. During discontinuous DF-NF hybrid process, 700 mL of RO water (flow rate of ~ 32 mL/minute) is introduced into the feed tank of green tea extract (3,500 mL) while permeate is removed and eliminated from the feed tank of green tea extract. RO water volume to feed/concentrate/retentate volume ratio is expressed as Number of Diavolume (N_d), i.e. 700 mL/3,500 mL (N_d 0.2). Discontinuous DF-NF hybrid process was stopped until collected permeate volume is equal with RO water volume introduced (Anonymous, 1981). The result of discontinuous DF-NF hybrid process was concentrate (retentate) with higher L-theanine content and permeate. This concentrate (retentate) is then introduced as a feed in further concentration process by means of NF membrane (Second Concentration Step). Pump motor frequency was adjusted in 25 Hz (flow rate ~ 7.5 L/minute) and operation pressure of 25 bar.

The same procedure was carried out for L-theanine-high concentrate (retentate) as a result of first concentration step. Pump motor frequency was adjusted in 25 Hz (flow rate ~ 7.5 L/minute) and operation pressure of 25 bar. Fluid passing via membrane pores area expressed in terms of permeate was collected into a mass cylinder to determine permeate flux value. When a stable flow was reached in module system, the samples in permeate and concentrate (retentate) were taken regularly and periodically, i.e. 0, 30, 60, 90, 120, and 150 minutes. A schematic flow diagram of the experimental set-up for NF concentration process of L-theanine from result of discontinuous DF-NF hybrid process as shown in Figure 2. Temperature in first concentration step, discontinuous DF-NF hybrid process and second concentration step were controlled and kept constant at room temperature (~ 23 – 25 °C) using chiller flowed by tap water (~ 23 – 24 °C).

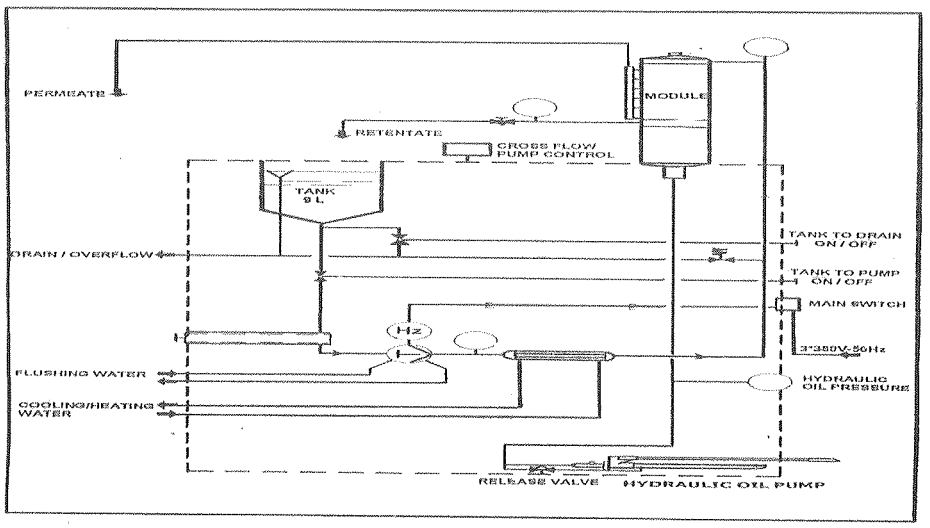

Figure 2. A schematic flow diagram of the experimental set-up for Nanofiltration process module (Anonymous, 1910)

RESULTS AND DISCUSSION

Characteristic of diafiltrate of green tea of Arraca yabukita grade

L-Theanine-high green tea is prepared through a series of processes, covering MF, NF, discontinuous DF-NF hybrid process and NF. In term of DF, Number of Diavolume (N_d) is expressed as RO water volume to feed/concentrate/retentate volume ratio, in which permeate is removed from the feed tank of green tea extract. In this process, 700 mL of RO water (flow rate ~ 32 mL/minute) is added into the feed tank of green tea extract (3,500 mL), i.e. 700 mL/3,500 mL (N_d 0.2) (Ghosh, R., 2003). Diafiltrate is brownish yellow suspension with contents of L-theanine, total solids, total polyphenols and dissolved protein of 5.1025 %, 0.1455 %, 14.65 % dan 1.5 mg/mL. respectively. The L-theanine and total polyphenols contents were higher than those in dry green tea leave (1 – 2 % and 10 – 15 %) (Liu, Z., 2006a). From this result showed that the main goal of L-theanine separation from other components in green tea extract, particularly total polyphenols was sufficient successfully when compared to L-theanine and total polyphenols in fresh green tea leave. Although, total polyphenol (14.65 %) in diafiltrate was still higher than L-theanine (5.1025 %). This matter is not possibility only caused by polyphenols compound domination in green tea extract with MW range of 200 – 600 Da., which is almost same with L-theanine MW (174 Da.), but also it is caused by intrinsic factors, such as higher solubility in water. Total polyphenol is an accumulation from a number of tea polyphenol, consisted of compound variety of flavonol (cathecin), flavones, anthocyanin and Leucoanthocyanidins, phenolic acids and depsides (Liu, Z., 2006b). Figure 3 displayed local dry green tea leave of *Arraca Yabukita* grade (a), green tea concentrate (retentate) as a result of NF process as feed of DF-NF hybrid process (b), and green tea concentrate as a result of DF-NF hybrid process as feed of NF process (c).

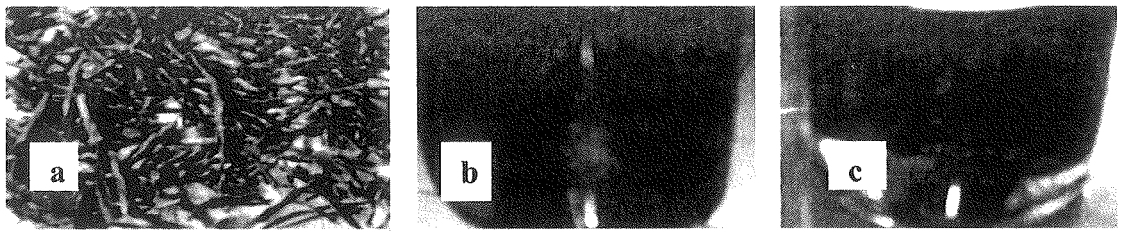

Figure 3. Local dry green tea of *Arraca yabukita* grade (a), green tea concentrate (retentate) of NF result as feed in DF-NF hybrid process (b), and green tea concentrate (retentate) of DF-NF hybrid process as NF process (c)

Effect of nanofiltration (NF) process on membrane performance Permeate Flux Value

Ideal membrane performances are high permeate flux value and high any component selectivity or rejection on membrane. The flux is expressed as an unit volume per unit membrane area per unit time interval, e.g., L/m^2.hour (Michael, A.S., 1989). Investigation result on permeate flux value at pump motor frequency of 25 Hz (7.5 L/minute), room temperature and operation pressure of 25 bar with interval time of each 30 minutes showed a drastically decrease of permeate flux value for 0 – 30 minutes and sufficient sharp drop of permeate flux value after 30 – 150 minutes, as shown in Figure 4.

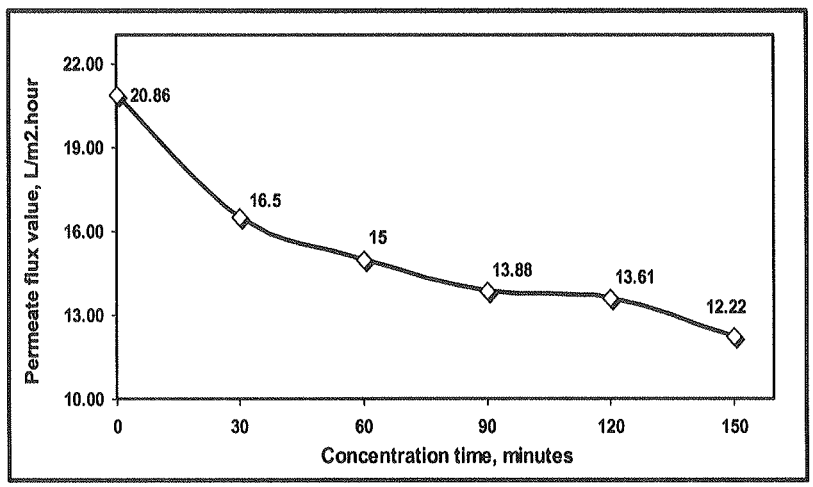

Figure 4. Effect of time on permeate flux value in concentration of green tea concentrate (retentate) of *Arraca Yabukita* grade as a result of DF-NF hybrid process

Initial sharp drop of permeate flux value is possibility caused by a fouling occurred firstly. After process of 30 minutes, permeate flux value changes enough slowly, that displayed deposition of solute particles on top active membrane surface. This condition causes its occurence of concentration polarization and increase of fluid viscosity as a consequency of pure solvent (water) mass transfer passing via membrane pores, so that it is happened formation of gel layer on top active membrane surface, that is expressed as concentration polarization (Cheryan, M., 1992; Mulder, M.H.V., 1996). Based on permeate flux value and rejection value of any component on membrane, the use of NF membrane indicated that NF membrane operates almost successfully. In other words, compounds in green tea extract having smaller particle size than pores size of NF

membrane (1 – 10 nm or MW less than 150 Da.) will pass freely in permeate. Green tea extract contain components of polyphenols/catechins (10 – 15 %/25 – 40 %), consisted of complex catechin, (–)-epigallocatechin gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG) and (–)-epicatechin (EC), L-Theanine (1 – 2 %), theaflavins (0,5 ~ 1 %), caffeine/ theobromine/ theopyllidyne (1 ~ 3 %), polysaccharides (1 ~ 2 %), saponin (0,5 ~ 1 %), flavoneglycoside < 0,5 %, anthocyanidins/ proanthocyanidins 1 – 2 % (Liu, Z., 2006b; Xiao, W., 2006) and element micro, such as selenium, iron and zinc, accumulated as total solids and effect of flow rate.

Effect of NF process on concentrate (retentate) and permeate compositions

L- theanine (%, dry weight basis)

Concentration process of green tea diafiltrate result of DF-NF hybrid process at pump motor frequency of 25 Hz (7.5 L/minute), room temperature and operation pressure of 25 bar for 0 – 150 minutes gave higher L-theanine content in concentrate (retentate) than that in permeate, as shown in Figure 5. In this operation condition for process of 0, 30, 60, 90, 120 and 150 minutes is produced L-theanine content in concentrate (retentate) of 5.0229 %, 4.2021 %, 5.3991 %, 5.6385 %, 6.0831 % and 7.2117 % (dry weight basis), respectively and in permeate of 0.0796 %, 0.0543 %, 0.0577 %, 0.0783 %, 0.109 % and 0.15 % (dry weight basis), respectively.

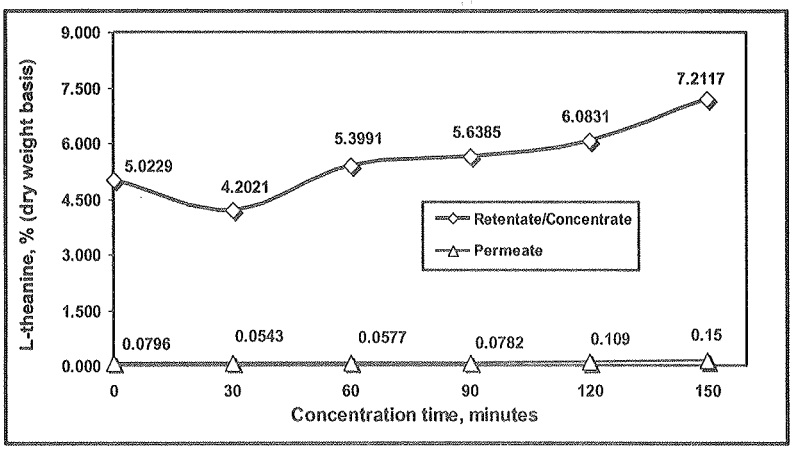

Figure 5. Effect of time on L-theanine content in concentrate (retentate) and permeate in concentration of green tea concentrate (retentate) of *Arraca Yabukita* grade as a result of DF-NF hybrid process

The long time of concentration process will increase L-theanine content in concentrate (retentate), and optimal time of concentration process is reached at 150 minutes (7.2117 %). While, for the same time of concentration process tends to be constant to end time of concentration process. Based on the highest content at optimal time, NF process increased concentration degree (CD) of L-theanine in concentrate (retentate) of 29.66 % from initial content (before process) of 5.1025 % (dry weight basis) to 7.2117 % (dry weight basis). L-theanine, like amino acids in general, has particles size ranging 0.01 – 0.1 µm with MW of 174, so that NF process will reject and retain more much L-theanine on top active membrane surface and less L-theanine passes only freely in

permeate. Because NF membrane has pore size of 0 – 10 nm, the utilize of NF membrane was able to get high rejection value (> 90 %) (Anonymous, 2010).

Dissolved Protein (mg/mL)

The similar trend seems for dissolved protein content, as demonstrated in Figure 6. The long time of concentration process would increase dissolved protein content in concentrate (retentate), but decreased dissolved protein content in permeate. At this operation condition, process of 0, 30, 60, 90, 120 and 150 minutes showed dissolved protein content in concentrate (retentate) of 1.5, 1.2, 1.5, 1.4, 2 and 2 mg/mL, respectively and in permeate of 0.007, 0.004, 0.004, 0.004, 0.002 and 0.005 mg/mL, respectively. Based on the highest dissolved protein content (2 mg/mL) at optimal time (150 minutes), NF process increased CD of dissolved protein in concentrate (retentate) of 25 % from initial content (before process) of 1.5 mg/mL to 2 mg/mL. Dissolved protein is an important indication of its presence of amino acids, such L-theanine and derivative or combination components between protein and polysaccharides as an enzymatic and non-enzymatic reactions for treating process of green tea, covering tea leave processes of whitering, steaming and drying.

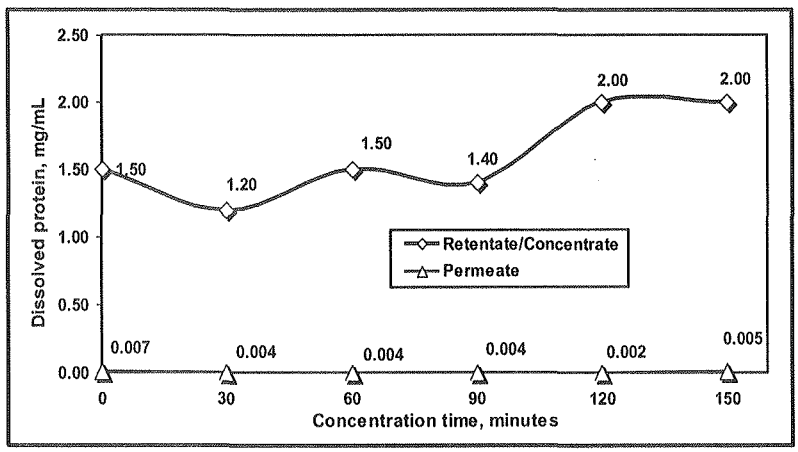

Figure 6. Effect of time on dissolved protein content in concentrate (retentate) and permeate in concentration of green tea concentrate (retentate) of *Arraca Yabukita* grade as a result of DF-NF hybrid process

During drying process, the tea leave is occurred Maillard reaction, in which polysaccharides of tea (1 – 2 %) and amino acids will produce derivative compounds, such as brown pigment of melanoidin (Belitz, H.D. and Grosch, W., 1999), that is a derivative compound yielded by reaction between peptides/protein and polysaccharides of green tea. With particle size ranging of 0.01 - 0.1 μm (Anonymous, 2005) or MW of 150 – 200 Da., the compounds in concentrate (retentate) (NF pore size of 0 – 10 nm) will be retained more much than pass freely in permeate. Amino acids tend to have high solubility in water (Belitz, H.D. and Grosch, W., 1999), so that extraction process via multifiltration influenced by operation condition (flow rate, temperature, turbulence, pressure and time) will be take placed a concentration polarization on top membrane

surface. For all processes, increase contents of L-theanine and dissolved protein in concentrate (retentate) was happened due to deficite of water mass passing freely through membrane pores, so that concentrate (retentate) fluid is thick. Cross-flow system flowing paralelly on top active membrane surface does not give an opportunity of particles accumulating on top active membrane surface, in which the solute particles will be back to feed/concentrate/retentate side, as a consequency, L-theanine and dissolved protein contents in feed/concentrate/retentate becomes more and more high. While, low contents of both components in permeate are occurred a result of interaction of protein particles (agregation) themselves and interactions amongst dissolved protein and L-theanine particles (adsorption), so that a part of particles with smaller particle size than NF membrane pores is able to pass freely via membrane pores.

Total Polyphenol (%, dry weight basis)

Total polyphenol is an accumulation of a number of green tea polyphenol, such as flavonol (cathecin), flavones, anthocyanin and leucoanthocyanidins, phenolic acids and depsides (Liu, Z., 2006a) with MW range of 200 – 600 g/mol. Difference in MW and solubility properties of L-theanine and total polyphenol in water cause the both components type are difficult to be separated. Concentration process of green tea diafiltrate gave higher content of total polyphenol in concentrate (retentate) when compared to permeate relating with long process time, as demonstrated in Figure 7.

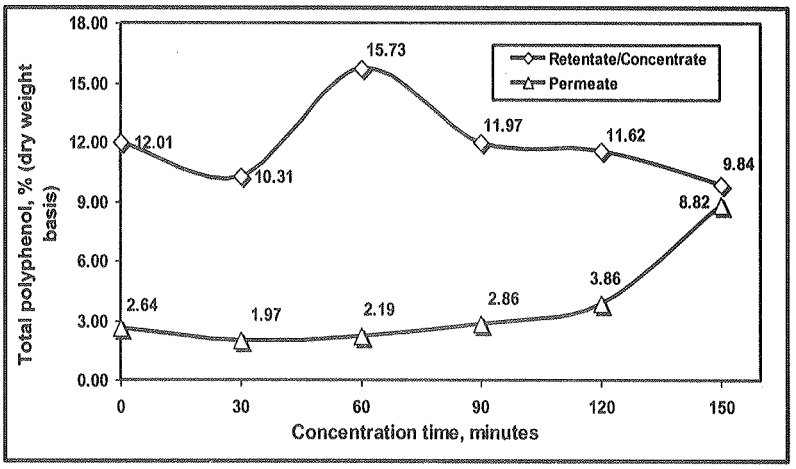

Figure 7. Effect of time on total polyphenol content in concentrate (retentate) and permeate in concentration of green tea concentrate (retentate) of *Arraca Yabukita* grade as a result of DF-NF hybrid process

Concentration process for 0, 30, 60, 90, 120 and 150 minutes result total polyphenol in concentrate (retentate) of 12.01 %, 10.31 %, 15.73 %, 11.97 % and 9.84 % (dry weight basis) and in permeate of 2.64 %, 1.97 %, 2.19 %, 2.86 %, 3.86 % and 8.82 % (dry weight basis). Concentration rate of total polyphenol in concentrate (retentate) fluctuates, in which concentration time of 60 minutes indicates the highest content of total polyphenol (15.73 %, dry weight basis) and becomes more and more low, whereas total polyphenol in permeate becomes more and more high to end process (8.82 %, dry weight basis). This condition is possibility caused by enough wide MW

range of total polyphenol (between 200 – 600 g/mol) with different chemical property and their interactions of operation condition (flow rate, temperature, pressure), so that NF system is still able to pass freely in permeate. At the end process (150 minutes), NF system is able to lower total polyphenol content in concentrate (retentate) of 32.63 % (dry weight basis) from initial content prior to process (14.65 %, dry weight basis) to 9.84 % (dry weight basis).

Total Solids (%, dry weight basis)

Different trend appears in total solids content, in which long concentration time will increase total solids content both in concentrate (retentate) and permeate, as displayed in Figure 8.

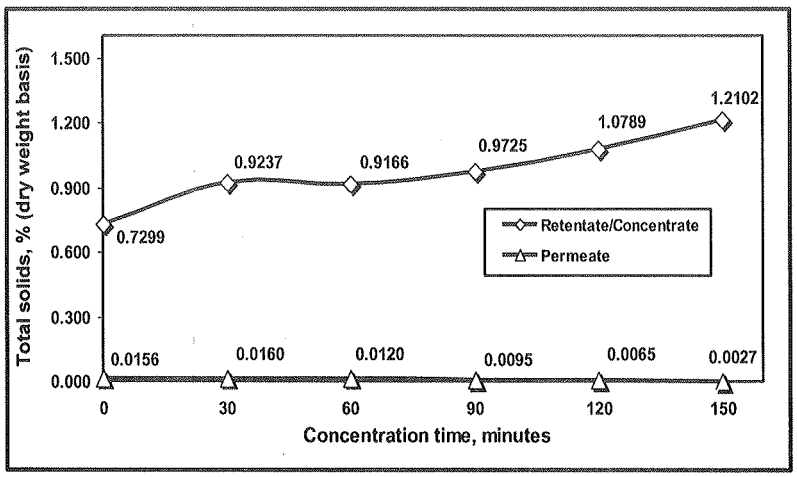

Figure 8. Effect of time on total solids content in concentrate (retentate) and permeate in concentration of green tea concentrate (retentate) of *Arraca Yabukita* grade as a result of DF-NF hybrid process

Concentration process time of 0, 30, 60, 90, 120 and 150 minutes results total solids content in concentrate (retentate) of 0.7299 %, 0.9237 %, 0.9166 %, 0.9725 %, 1.0789 % and 1.2102 % (dry weight basis) and in permeate of 0.0156 %, 0.016 %, 0.012 %, 0.0095 %, 0.0065 % and 0.0027 % (dry weight basis), respectively. Total solids are all green tea components affecting on fluid flow rate and ability of membrane to separate components. Green tea extract contain all tea components both dissolved and non-disolved. The main components in green tea are dominated by polyphenols/cathecins (10 – 15 %/25 – 40 %), L-theanine (1 – 2 %), theaflavins (0.5 ~ 1 %), caffeine/theobromine/theaphylline (1 ~ 3 %), polysaccharides (1 ~ 2 %), saponins (0.5 ~ 1 %), flavoneglycosida (< 0.5 %) and anthocyanidin/ proanthocyanidin (1 – 2 %), and minerals (Calcium, Magnesium, Zinc) (Gong, Y, *et al.*, 2006), that contributes on total solids. NF membrane system yields total solids in concentrate (retentate), which becomes more and more high, but total solids in permeate tends to be constant relating with its long concentration time. Optimal concentration time of total solids in concentrate (retentate) (1.2102 %) and in permeate (0.0027 %) was reached in 150 minutes. Based on the highest total solids content at the optimal time, NF membrane system increases total solids content of 87.58 % from initial content prior to process (0.1455 %, dry weight basis) to 1.2102 % (dry weight basis).

Rejection Coefficient (%)

One of the important performance parameters of NF membrane system in concentration process of L-theanine in green tea extract is rejection (R) of any component on membrane. The rejection (R) of any component is defined as $R = [C_f - C_p]/C_f$, where C_f and C_p are the content of component in the feed and the permeate, respectively. The rejection is determined experimentally for each component in the feed, by the sampling the feed and permeate at the same time and analysing that component. Rejection values can be used to check the ability, integrity and performance of a membrane (Cheryan, M., 1992; Mulder, M.H.V., 1996). Figure 9 showed the effect of concentration time of green tea of *Arraca Yabukita* grade on L-theanine and dissolved protein rejections as a result of NF process at pump motor frequency of 25 Hz (7.5 L/minute), room temperature and operation pressure of 25 bar.

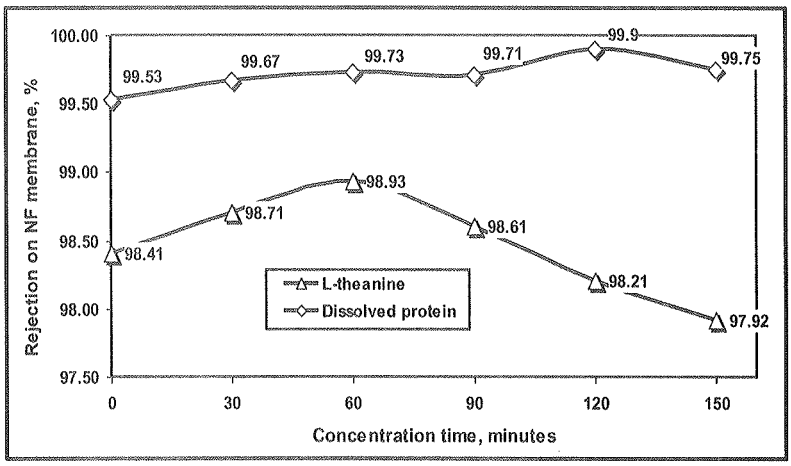

Figure 9. Effect of time on L-theanine and dissolved protein rejections in concentration of green tea concentrate (retentate) of *Arraca Yabukita* grade as a result of DF-NF hybrid process

Concentration process time of 0, 30, 60, 90, 120 and 150 minutes gave L-theanine rejection of 98.91 %, 98.71 %, 98.93 %, 98.61 %, 98.21 %, and 97.92 %, respectively, whereas dissolved protein rejection for the same time were 99.53 %, 99.67 %, 99.73 %, 99.71 %, 99 % and 99.75 %, respectively. Based on the rejection value, selectivity of NF membrane on L-theanine and dissolved protein components are almost succesfully, due to approximately ~ 100 %, so that NF membrane is able to separate L-theanine and dissolved protein components in green tea extract from other components. This matter is not caused only by particle size of L-theanine and dissolved protein ranging of 0.01 - 0.1 μm with MW of L-theanine of 174 Da. and MW of dissolved protein ranging of 150 – 200 Da., but also by using NF membrane, which enables to be resulted rejection more than 99 %. Based on NF membrane performance, optimal concentration time of green tea diafiltrate was reached at 60 minutes with separation selectivity of L-theanine of 98.93 %. At this condition, L-theanine and dissolved protein contents in concentrate (retentate) are 5.39915 % and 1.5 mg/mL, respectively. Whereas, based on the highest content of L-theanine, optimal time is reached at 150 minutes with L-theanine content of 7.2117 % and dissolved protein content of 2

mg/mL. Difference in this condition is possibility caused by its presence of other components with similar MW (polyphenols, etc), causing a shearing from optimal membrane performance, besides sensitivity of NF membrane.

Figure 10 demonstrated the effect of concentration time of green tea of *Arraca Yabukita* grade on total solids and total polyphenol rejections as a result of NF membrane system at pump motor frequency of 25 Hz (7.5 L/minute), room temperature and operation pressure of 25 bar. At this condition, concentration process time of 0, 30, 60, 90, 120 and 150 minutes gave total solids rejection of 78.01 %, 80.94 %, 86.07 %, 70.76 %, 66.74 % and 10.38 %, respectively, whereas total polyphenol rejection for the same time were 97.87 %, 98.27 %, 98.7 %, 99.02 %, 99.4 % and 99.78 %, respectively. It is seen that total polyphenol component showed low rejection value because rejection is only ranging 10.38 - 86.07 % or less than 99 %. This matter is possibility caused by various polyphenol MW (> 6 compounds) of 200 - 600 Da., so that ratio of total polyphenols content in concentrate (retentate) and total polyphenol content in permeate for optimal process time (150 minutes) is equal.

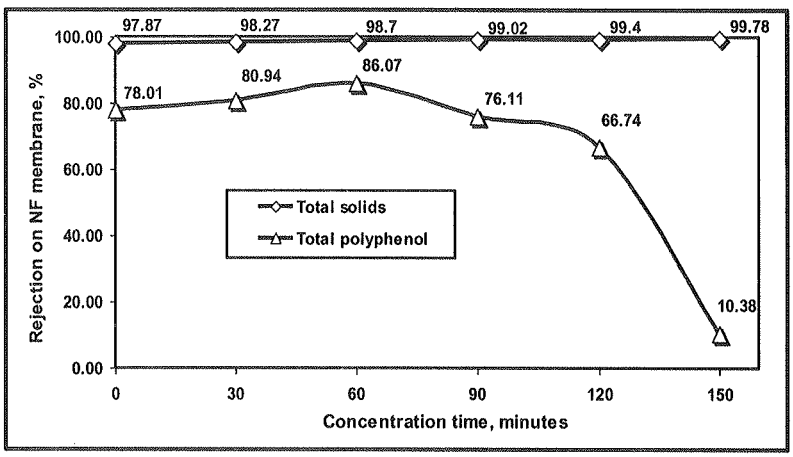

Figure 10. Effect of time on total solids and total polyphenol rejections in concentration of green tea concentrate (retentate) of *Arraca Yabukita* grade as a result of DF-NF hybrid process

It is known that total polyphenol MW ranging 200 – 600 Da. is consisted of accumulation of a number of green tea polyphenols, covering compounds of flavonol (cathecin), flavones, anthocyanin & leucoanthocyanidins, phenolic acids & depsides (Liu, Z., 2006a). Due to effect of pressure and time process, a part of total polyphenol will pass freely in permeate, and is retained in concentrate (retentate). This situation causes ratio of components passed and retained are almost similar, so that selectivity of membrane is low. Different condition seems at total solids, in which ratio of retained component in concentrate (retentate) and passed component in permeate is high, so that membrane selectivity is high and rejection value is range of 97.87 - 99.78 % or more than 99 % or almost perpect (~ 100 %). The highest rejection value is occurred for process of 150 minutes (99.78 %), in which at this condition, optimal content of total solids is 1.2102 % (dry weight). Based on the highest total content at optimal time, NF membrane system increases total solid content from initial content prior to process (0.7299 %, dry weight) to 1.2102 % (dry weight).

Based on the highest L-theanine content (7.21 %, dry weight basis) in concentrate (retentate), optimal time in NF process at pump motor frequency of 25 Hz (7.5 L/minute), room temperature and operation pressure of 25 bar for 0, 30, 60, 90, 120 and 150 minutes was reached at 150 minutes. At the same time, it is yielded total polyphenol content of 9.84 %. Concentration process by means of NF membrane increased L-theanine content in concentrate (retentate) of 30.37 %, from initial time (5.02 %) to 150 minutes (7.21 %), but decreased total polyphenols content of 24.73 % from initial process time (12.01 %) to process time of 150 minutes (9.04 %). NF membrane system passes freely L-theanine in permeate of 0.15 % (dry weight basis) and total polyphenols of 8.82 % (dry weight basis) reached at optimal operation condition (150 minutes). Figures 11a and 11b showed green tea concentrate (retentate) of brownish thick suspension and permeate of clear liquid.

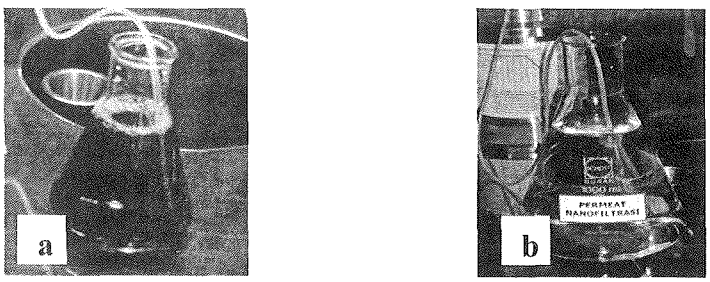

Figure 11. Concentrate (retentate) (a) and permeate (b) as a result of concentration process of green tea diafiltrate of *Arraca Yabukita* grade via NF membrane at pump motor frequency of 25 Hz (7.5 L/minute), room temperature (~ 23 - 25 °C) and operation pressure of 25 Bar for 150 minutes

CONCLUSION

The experiment result showed that NF membran system was able to separate L-theanine successfully, in which L-theanine was rejected and retained more much in concentrate (retentate) than that passing freely in permeate. The long NF process time would drop permeate flux value, but increased L-theanine, total polyphenol, dissolved protein and total solids contents. Based on the highest L-theanine content, optimal concentration time to produce concentrate (retentate) was reached at 150 minutes with contents of L-theanine, total polyphenol, dissolved protein, and total solids of 7.2117 %, 9.84 %, 2 mg/mL, and 1.2102 %, respectively.

At the same time, NF membrane system passes still L-theanine, total polyphenol, dissolved protein, and total solids in permeate of 0.15 %, 8.82 %, 0.005 mg/mL, and 0.0027 %, respectively, with permeate flux value of 12.22 L/m^2.hour. Based on the highest content at optimal time, NF membrane system is able to increase concentration degree of L-theanine, dissolved protein, and total solids in concentrate (retentate) of 29.66, 25 and 87.58 %, but decreases total polyphenol of 32.63 % from before and after concentration process.

Selectivity of NF membrane on L-theanine, dissolved protein, total polyphenol and total solids were 97.92 %, 99.75 %, 10.38 %, and 99.78 %, respectively. Permeate as a result of NF process has a potential value as functional drink for relaxation situation.

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