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Improvement of purification process of stevia extract by combination of microfiltration and ultrafiltration

Peningkatan proses pemurnian ekstrak stevia menggunakan kombinasi mikrofiltrasi dan ultrafiltrasi

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Abstrak

Mikrofiltrasi dan ultrafiltrasi digunakan pada proses pemurnian ekstrak stevia untuk mempertahankan steviosida dan menghilangkan tanin. Tujuan utama penelitian ini adalah mendapatkan kondisi operasi proses pemurnian ekstrak stevia yang menghasilkan rejeksi steviosida terendah dan rejeksi tanin tertinggi. Proses pemurnian ekstrak stevia menggunakan membrane mikrofiltrasi dilaku-kan pada tekanan trans-membran (1,20; 1,40; 1,65; 1,80; dan 1,90 bar), kecepatan alir (0,04, 0,06, dan 0,11 m det^{-1}), dan konsentrasi steviosida umpan (7,12; 10,25; 14,03; dan 18,47 g L^{-1}). Proses pemurnian ekstrak stevia menggunakan membran ultrafiltrasi pada tekanan transmembrane (1,20; 1,40; 1,65; 1,80; dan 1,90 bar), kecepatan alir (0,06; 0,09; dan 0,12 m det^{-1}), dan konsentrasi steviosida umpan (4,59 dan 10,36 g L^{-1}). Proses pemurnian tahap pertama dilakukan menggunakan membran mikrofiltrasi dan hasil permeatnya sebagai umpan proses ultrafiltrasi. Proses pemurnian tahap kedua dilaku-kan menggunakan membran ultrafiltrasi. Kondisi operasi terbaik proses mikrofiltrasi menggunakan konsentrasi steviosida umpan 14,03 g L^{-1} pada tekanan transmembran 1,90 bar dan kecepatan alir 0,11 m det^{-1} dengan fluksi permeat 82,90 L m^{-2} jam^{-1}. Kondisi operasi terbaik proses ultrafiltrasi menggunakan konsentrasi steviosida umpan 10,36 g L^{-1} dengan fluksi permeat 26,51 L m^{-2} jam^{-1} pada tekanan transmembran 1,90 bar dan kecepatan alir 0,12 m det^{-1}. Proses mikrofiltrasi dan ultrafiltrasi menghasilkan total rejeksi steviosida 59,52 % dan total rejeksi tanin 57,99 %.

[Kata kunci: mikrofiltrasi, proses pemurnian, steviosida, ultrafiltrasi]

Abstract

Microfiltration and ultrafiltration are used for the purification process of stevia extract to retain steviosides and remove tannins. The main objective of this study was to obtain the operating conditions for the purification process of stevia extract that resulted in the lowest stevioside rejection and highest tannin rejection. The purification process of stevia extract using microfiltration membrane was carried out at transmembrane pressure (1.20, 1.40, 1.65, 1.80, and 1.90 bar), cross flow velocity (0.04, 0.06, and 0.11 m s^{-1}), and stevioside concentration of feed (7.12, 10.25, 14.03, and 18.47 g L^{-1}). The stevia extract purification process used ultrafiltration membrane at transmembrane pressure (1.20, 1.40, 1.65, 1.80, and 1.90 bar), cross flow velocity (0.06, 0.09, and 0.12 m s^{-1}), and stevioside concentration of feed (4.59 and 10.36 g L^{-1}). The first step purification process was carried out using a microfiltration membrane and the resulting permeate was used as feed for the ultrafiltration process. The second step purification process was carried out using an ultrafiltration membrane. The best operating conditions of the microfiltration process were feed stevioside concentration of 14.03 g L^{-1} at a transmembrane pressure of 1.90 bar and a cross flow velocity of 0.11 m s^{-1} with a permeate flux of 82.90 L m^{-2} h^{-1}. The best operating conditions of the ultrafiltration process used a feed stevioside concentration of 10.36 g L^{-1} with a permeate flux of 26.51 L m^{-2} h^{-1} at a transmembrane pressure of 1.90 bar and a cross flow velocity of 0.12 m s^{-1}. The microfiltration and ultrafiltration processes resulted in total stevioside rejection of 59.52 % and total tannin rejection of 57.99 %.

[Keywords: microfiltration, purification process, stevioside, ultrafiltration]

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Introduction

Natural sweetener from stevia leaves are very potential sweetener with high commercialization value because it contains steviol glycosides (SGs) which provide sweet taste. Steviol glycosides are classified as low-calorie or non-calorie sweeteners with sweetness level 300 times sweeter than sucrose (Basharat et al., 2021). Steviol glycosides present in stevia leaves include stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, dulcoside A, and steviolbioside (Lemus-Mondaca et al., 2012). The main compounds of steviol glycoside contained in stevia leaves are stevioside around 4-13 % and rebaudioside A around 2-4 % (Kurek & Krejpcio, 2019). There are other constituents found in stevia leaves, such as tannins, chlorophyll, carotenoids, and polyphenols.

Stevia leaf sweetener does not cause chronic disease problems such as diabetes mellitus, fatty liver, hypertension, cardiac fibrosis, liver fibrosis, inflammatory bowel disease, cancer, and chronic kidney disease (Ahmad et al., 2020; Wang et al., 2020). Stevia leaves are in great demand as sweeteners, pharmaceuticals, and food additives in the global market. The conventional process for extracting stevia leaves is generally carried out by using solvents. Still, this process requires a long time, high energy consumption, lots of solvents, and high solvent losses (Kovačević et al., 2018). Various methods can be used for extraction, recovery, or purification of stevia extracts, such as clarification with a chelating agent (Mantovaneli et al., 2004), ion exchange resin (Abou-Arab et al., 2009), high-speed countercurrent chromatography (Huang et al., 2010), microwave-assisted extraction (Jaitak et al., 2009), ultrasonication-assisted extraction (Gasmalla et al., 2017), centrifugal partition chromatography (Hubert et al., 2015), and column chromatography (Kaur et al., 2014).

Membrane technology can be used for the purification process of stevia extract, for example, the microfiltration process with a pore size of 0.2 μm made from PES under operating condition of 1.38 bar transmembrane pressure produces stevioside of 89.1 %, but the total solids obtained are still quite high at 84.38 % (Chhaya et al., 2013) and ceramic membrane microfiltration process with a pore size of 0.05 μm with a transmembrane pressure of 2 bar can produce stevioside by 97.1 % and rebaudioside A by 94.7 % (Reis et al., 2009). Purification of stevia extract by ultrafiltration process made from PES MWCO 20 kDa with transmembrane pressure of 1.87 bar and cross flow velocity of 0.02 m s^{-1} resulted in the lowest stevioside rejection of 36 % (Noor & Isdianti, 2007), and ultrafiltration process made from PES MWCO 30 kDa with transmembrane pressure of 2.76 bar and cross flow velocity of 0.1 m s^{-1} resulted in stevioside of 71.8 % with total solid of 62.96 % (Chhaya et al., 2012). Purification of stevia extracts with two-step ultrafiltration process (100 kDa and 1 kDa) made from PS can remove total solids by 97.58 % and carbohydrates by 98.11 % with a concentration of rebaudioside A of 6.12 % in permeate (Díaz-Montes et al., 2020). The nanofiltration process made from polyamide-coated polysulfone MWCO 400 Da with a transmembrane pressure of 11.03 bar and a stirring speed of 1500 rpm can produce stevioside of 7.1 % with clarity of 98.1 %T (Chhaya et al., 2012) and the nanofiltration process of MWCO 200 Da with a pressure of 30.5 bar produces stevioside of 2.8 % and rebaudioside A of 1.3 % (Kootstra et al., 2015).

Membrane technology has advantages over other separation processes, such as low cost, low energy requirements, high separation efficiency, and easy to connect with other separation processes (Purkait et al., 2018). In general, the efficiency of membrane performance can be measured from the flux and rejection values of the membrane. In addition, permeate purity is also an important parameter that must be considered to measure membrane performance. The phenomena of fouling and concentration polarization often occur in membrane processes that cause a decrease in product accumulation due to a decrease in flux. High operating pressure can increase permeate flux, but it can also aggravate fouling so proper operating pressure is needed to control fouling and flux (Wei et al., 2021). The cross flow mode can reduce the thickness of the cake layer on the membrane surface due to turbulence and an increase in the mass transfer coefficient which can minimize particle deposition in the cake layer so as to increase membrane permeability (Zhang et al., 2019). Based on the above description, it is necessary to obtain membrane process operating conditions that produce high separation efficiency and high flux, as well as determine the effect of operating conditions on permeate flux, membrane rejection, and properties of permeate. Therefore, the stevia extract purification process can be carried out using combination microfiltration and ultrafiltration membrane cross flow mode with operating conditions of transmembrane pressure, cross flow velocity, and feed concentration. The main objective of this study was to obtain the operating conditions for purification process of stevia extract that produce the lowest stevioside rejection and the highest tannin rejection.

Materials and Methods

Materials

The main ingredients used were stevia leaves obtained from a farm in Katulampa, East Bogor. Distilled water was used as solvent for extraction process. Standard stevioside of 95 % purity from Sigma-Aldrich.

Preparation of stevia extract

Fresh stevia leaves were dried in an oven blower at 60 °C for ± 3-5 hours. After the drying process, the size reduction stage was continued into stevia powder using blender, then filtered using 80 mesh sieve. Four stevia extracts were used as microfiltration feed with ratio of stevia powder and distilled water 1:100 (w/v), 1:75 (w/v), 1:60 (w/v), and 1:50 (w/v). According to Chhaya et al. (2012), extraction process used maceration method carried out at 78 ± 1 °C for 56 min, then the crude stevia extract was filtered using filter cloth and centrifuged at 6000 rpm for 10 min. The stevia extract obtained was analyzed before the purification process to determine the initial characteristics such as stevioside, tannin, total sugar, color, and clarity.

Stevia extract purification process using microfiltration and ultrafiltration membranes

The initial condition of the membrane was observed before the filtration process by determining the water flux which was done by recirculating distilled water at 25 ± 1 °C for 30 min. The water flux permeability of the microfiltration process was carried out under operating conditions at a transmembrane pressure of 1.90 bar and a cross flow velocity of 0.11 m s^{-1}, while the ultrafiltration process was carried out under the operating conditions at a transmembrane pressure of 1.90 bar and a cross flow velocity of 0.12 m s^{-1}. The steady state was determined by recirculating the stevia extract at 40 ± 1 °C for 30 min until it reached steady state. The purification process of stevia extract using microfiltration and ultrafiltration membranes was carried out to observe the effect of the operating conditions on flux, rejection, and properties of permeate. The purification process of stevia extract using microfiltration was carried out at five levels of transmembrane pressure (1.20, 1.40, 1.65, 1.80, and 1.90 bar), three levels of cross flow velocity (0.04, 0.06, and 0.11 m s^{-1}), and four levels of stevioside concentration of feed (7.12, 10.25, 14.03, and 18.47 g L^{-1}). Purification process of stevia extract using ultrafiltration at five levels of transmembrane pressure (1.20, 1.40, 1.65, 1.80, and 1.90 bar), three levels of cross flow velocity (0.06, 0.09, and 0.12 m s^{-1}), and two levels of stevioside concentration of feed (4.59 and 10.36 g L^{-1}). The first step purification process was conducted using a microfiltration membrane to obtain the best process operating conditions and permeate as feed for the ultrafiltration process. The second step purification process was carried out using an ultrafiltration membrane to obtain the best process operating conditions. The effectiveness of the purification process was determined by the flux and rejection of steviosides and tannins. The best operating of purification process was determined by total sugar increase, color reduction, and permeate clarity increase.

Stevioside analysis

The analysis was conducted based on a modified method of Noor & Isdianti (2007). A standard curve was prepared using 0.02 grams of stevioside standard in 100 mL of distilled water, then diluted. The solution was measured using a UV spectrophotometer at a wavelength of 210 nm. The absorbance reading of the samples was assessed by diluting the samples at the absorbance range of the standard curve. The stevioside concentration of the sample can be measured by calculating the absorbance from the standard curve regression equation $y = 47.979x + 0.0819$ with $R^2 = 0.995$, then multiplying by the dilution factor.

Tannin analysis

The analysis was performed based on the method of Kusumaningsih et al. (2015). The standard curve was prepared using 1 mL of tannin standard in a 10 mL volumetric flask, then 7.5 mL of distilled water was added. Folin ciocalteu as much as 0.5 mL was added to 1 mL of saturated Na_2CO_3, then diluted using distilled water. The solution was mixed until color formed, then waited for 30 min and analyzed using a UV-Vis spectrophotometer at a wavelength of 760 nm. The reading of the absorbance value of the sample is done by diluting the sample to the absorbance range of the standard curve. The tannin concentration of the sample can be measured by calculating its absorbance from the standard curve regression equation $y = 8.5609x + 0.0021$ with $R^2 = 0.998$, then multiplying by the dilution factor.

Total sugar analysis with phenol-sulfate method

Analysis followed a modified method of Noor & Isdianti (2007). Sample solution 2 mL (containing 20-50 ppm glucose) was added to 1 mL of 5 % phenol solution, then 5 mL of concentrated H_2SO_4 was added, and the solution was allowed to cool. The absorbance value of the sample was measured using a UV spectrophotometer with a wavelength of 490 nm. The total sugar concentration value can be measured by calculating the absorbance from the standard curve regression equation $y = 112.74x - 0.029$ with $R^2 = 0.995$.

Color analysis

The analysis was conducted based on the method of Chhaya et al. (2012). Color measurements were made using a spectrophotometer by calculating the absorbance of the sample (A) at a wavelength of 420 nm.

Clarity analysis

The analysis was conducted based on the method of Chhaya et al. (2012). The clarity value of the sample was measured using a spectrophotometer with the equation $\%T = 100 \times 10^{-A}$, where A is the the optical absorbance at a wavelength of 660 nm.

Data analysis

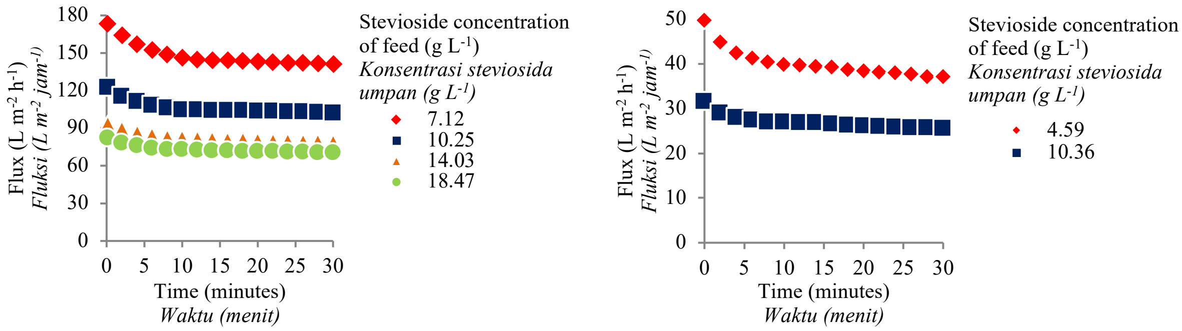
The experimental design used was a completely randomized design which was arranged factorially with two replications. The observed factor was the interaction effect of the operating conditions of the purification process on the properties of permeate. Data obtained from the results of the permeate test were analyzed using analysis of variances at significant level (α) of 5 % using the SPSS 20 program to determine whether there were differences between treatment combinations. If the results were significantly different, the analysis would be continued with a Duncan's multiple range test.

Results and Discussion

Steady state

The experiment was carried out using total recycle mode by flowing the permeate and retentate back to the feed tank and the feed concentration was kept constant. The operating conditions of the microfiltration and ultrafiltration processes, as well as the relationship between flux and filtration time can be seen in Figure 1. The flux produced by the microfiltration process is higher because the pore size of the microfiltration membrane is larger than that of the ultrafiltration membrane. The steady state of the microfiltration process was reached after 10 – 12 min and the steady state of the ultrafiltration process was reached after 10 min (Fig. 1). The permeate flux decreases with operating time until steady conditions are reached, this phenomenon often occurs due to the blockage of the membrane pores with larger compounds which can precipitate in the pores so that they can form a cake layer which results in decreased membrane permeability (Díaz-Montes et al., 2020).

The reversible buildup of particles from components in the feed can cause an increase in resistance due to the formation of fouling and concentration polarization (Tomczak & Gryta, 2020). Components with molecular weight lower than stevioside are categorized in low molecular weight (LMW) solutes and high molecular weight (HMW) components can be retained by the membrane, however some stevioside can also be retained by the gel layer and membrane. During the membrane process, feed components may adsorb on the pore or within the membrane pore, thus partially or completely closing the pore which may cause fouling and concentration polarization (Castro-Muñoz et al., 2021; Chhaya et al., 2012). The time when the resulting flux was constant in the microfiltration process and the ultrafiltration process was used as the steady time for the stevia extract purification process under operating conditions of transmembrane pressure, cross flow velocity, and stevioside concentration of feed.



(a) (b)

Figure 1. The relationship between flux and filtration time (a) the microfiltration process on transmembrane pressure at 1.90 bar and cross flow velocity at 0.11 m s^{-1}, and (b) the ultrafiltration process on transmembrane pressure at 1.90 bar and cross flow velocity at 0.12 m s^{-1} with different stevioside concentrations of feed

Gambar 1. Hubungan antara fluksi dan lama filtrasi (a) proses mikrofiltrasi pada tekanan transmembran 1,90 bar dan kecepatan alir 0,11 m det^{-1}, serta (b) proses ultrafiltrasi pada tekanan transmembran 1,90 bar dan kecepatan alir 0,12 m det^{-1} dengan konsentrasi steviosida umpan yang berbeda-beda

Cross flow microfiltration

The first stage of the stevia extract purification process was carried out using microfiltration membrane. Graph of the relationship between the operating conditions of the microfiltration process and permeate flux can be seen in Figure 2. The results show that higher transmembrane pressure and cross flow velocity increased permeate flux, while higher stevioside concentration of feed decreased permeate flux. According to Chhaya et al. (2013), permeate flux increases when the transmembrane pressure is increased. Transmembrane pressure can be driving force for selective membrane separation (Castro-Muñoz et al., 2022). According to (Reis et al., 2009) transmembrane pressure can be used to help steviosides pass through the membrane. The highest permeate flux was obtained using feed with stevioside concentration of 7.12 g L^{-1} on transmembrane pressure at 1.90 bar.

By increasing the cross flow velocity, particles can diffuse back into more significant suspension so that cake layer will be formed thinner on the membrane surface (Kazemi et al., 2013). The highest permeate flux was obtained using feed with stevioside concentration of 7.12 g L^{-1} on cross flow velocity at 0.11 m s^{-1}. When solidification of the cake layer occurs continuously on the membrane due to high transmembrane pressure, only the cross flow velocity can be optimized (Zhang et al., 2019). Foulant can flow on the membrane surface when the drag force by the cross flow velocity is greater than the tangential force to the membrane surface (Gao et al., 2019). According to Noor & Isdianti (2007) higher feed concentration will increase the viscosity, which can trigger fouling and concentration polarization resulting in decreased permeate flux.

Overall, the rejection of stevioside and tannins in the microfiltration process fluctuated relatively, as can be seen in Figure 3 and Figure 4. The results of the permeate characteristics analysis showed that the microfiltration membrane could reject stevioside and tannin. Stevioside rejection rates ranged from 26.17 % - 61.06 %, and tannin rejection rates ranged from 12.77 % - 68.75 %. High transmembrane pressure can result in the deposition of solutes on the membrane surface to form cake layer which acts as dynamic membrane to hold stevioside so that its concentration can be reduced in the permeate (Chhaya et al., 2013).

The total sugar content produced also relatively fluctuated with increase in transmembrane pressure and cross flow velocity, the highest total sugar content was produced using bait with stevioside concentration of 18.47 g L^{-1}. (Noor & Isdianti, 2007) reported that the decreased total sugar content indicated that the membrane process could retain sugars other than steviol glycosides. Increasing transmembrane pressure and cross flow velocity can reduce color and increase clarity, but higher feed concentrations resulted in stronger color and lower clarity of permeate. The selected permeate with the lowest stevioside rejection will be used as feed for the ultrafiltration process.

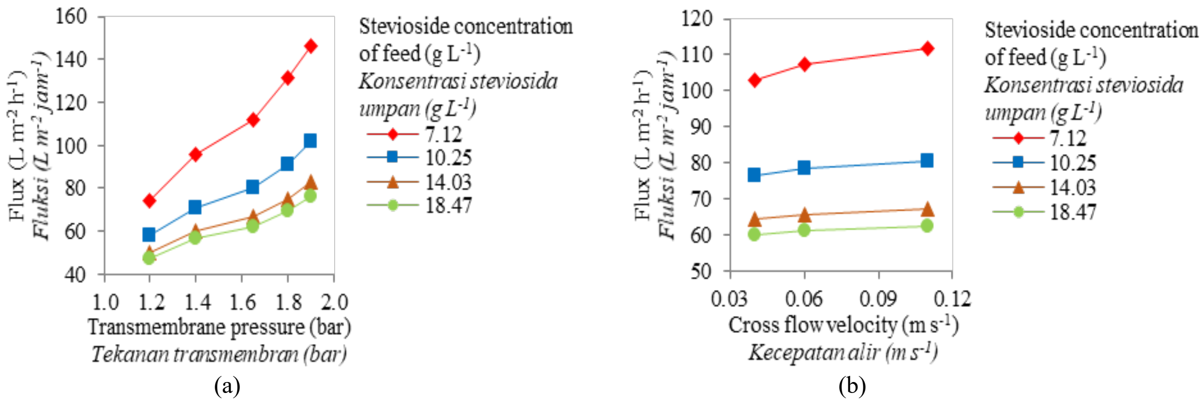
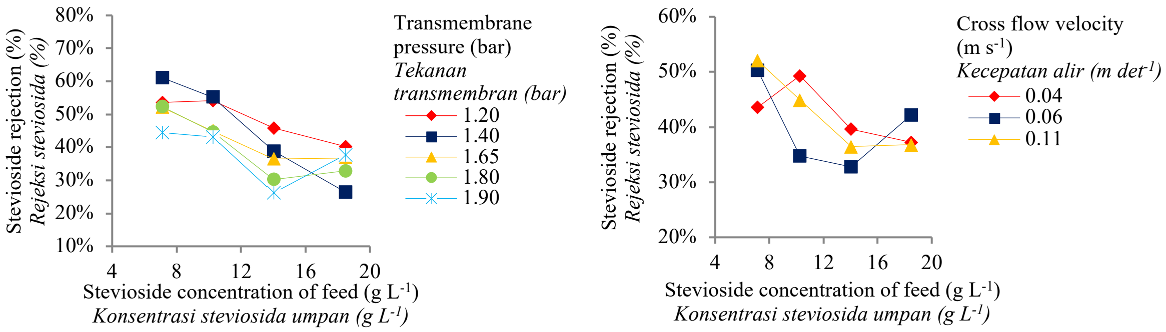


Figure 2. Graph of the relationship between (a) transmembrane pressure to permeate flux on cross flow velocity at 0.11 m s^{-1} and (b) cross flow velocity to permeate flux on transmembrane pressure at 1.65 bar with different stevioside concentrations of feed

Gambar 2. Grafik hubungan antara (a) tekanan transmembran terhadap fluksi permeat pada kecepatan alir 0,11 m det^{-1} dan (b) kecepatan alir terhadap fluksi permeat pada tekanan transmembran 1,65 bar dengan konsentrasi steviosida umpan yang berbeda-beda

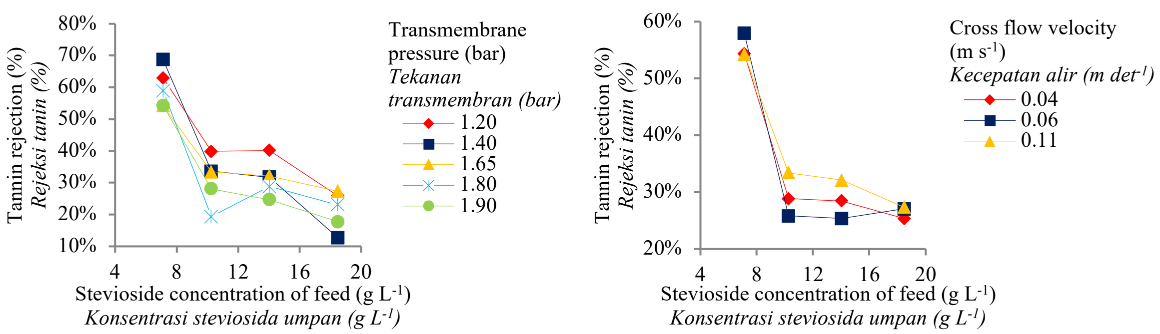
There were two permeates used as feed for the ultrafiltration process, namely comparison permeate and selected permeate. The comparison permeate was obtained using feed with stevioside concentration of 10.25 g L^{-1} on transmembrane pressure at 1.40 bar and cross flow velocity at 0.11 m s^{-1}. The selected permeate was obtained on transmembrane pressure at 1.90 bar and cross flow velocity at 0.11 m s^{-1} using feed with stevioside concentration of 14.03 g L^{-1} with permeate flux of 82.90 L m^{-2} h^{-1} which resulted in the lowest stevioside rejection of 26.17 % with tannin rejection 24.66 %, total sugar content 76.70 %, color reduction 57.86 %, and clarity 78.70 % T. The results of analyzing the properties of the permeate microfiltration process at different transmembrane pressures and stevioside concentrations of feed with a cross flow velocity of 0.11 m s^{-1} can be seen in Table 1. The properties of the permeate microfiltration process at different cross flow velocities and stevioside concentration of feed with transmembrane pressure of 1.65 bar can be seen in Table 2. Analysis of variances showed that the interaction between the stevioside concentration of feed (7.12, 10.25, 14.03, and 18.47 g L^{-1}) with transmembrane pressure (1.20, 1.40, 1.65, 1.80, and 1.90 bar) and cross flow velocity (0.04, 0.06, and 0.11 m s^{-1}) had significant effect on stevioside, tannins, total sugar, color, and clarity of the permeate. The microfiltration process can remove tannins and total solids with increased permeate clarity. The results of the purification process showed that stevia extract clarification using microfiltration membrane was quite effective in removing non-steviol glycoside compounds.



(a) (b)

Figure 3. Graph of the relationship between (a) transmembrane pressure and (b) cross flow velocity on stevioside rejection in the microfiltration process

Gambar 3. Grafik hubungan antara (a) tekanan transmembran dan (b) kecepatan alir terhadap rejeksi steviosida pada proses mikrofiltrasi



(a) (b)

Figure 4. Graph of the relationship between (a) transmembrane pressure and (b) cross flow velocity on tannin rejection in the microfiltration process

Gambar 4. Grafik hubungan antara (a) tekanan transmembran dan (b) kecepatan alir terhadap rejeksi tanin pada proses mikrofiltrasi

Table 1. Analysis of properties of permeate microfiltration process at different transmembrane pressures and stevioside concentration of feed with cross flow velocity of 0.11 m s^{-1}

Tabel 1. Analisis karakteristik permeat proses mikrofiltrasi pada berbagai tekanan transmembran dan konsentrasi steviosida umpan dengan kecepatan alir 0,11 m det^{-1}

Feed concentration *Konsentrasi umpan*	Transmembrane pressure *Tekanan transmembran* (bar)	Stevioside *Steviosida* (g L^{-1})	Tannin *Tanin* (g L^{-1})	Total sugar *Total gula* (g L^{-1})	Color *Warna* (A)	Clarity *Kejernihan* (%T)
	1.20	3.31 ± 0.92^{lm}	2.94 ± 0.06^{k}	1.07 ± 0.04^{hi}	0.639 ± 0.001^{i}	83.66 ± 1.23^{c}
	1.40	2.77 ± 0.57^{m}	2.49 ± 0.24^{l}	0.79 ± 0.01^{i}	0.631 ± 0.004^{i}	86.40 ± 0.70^{b}
I	1.65	3.41 ± 0.84^{lm}	3.64 ± 0.06^{j}	1.36 ± 0.01^{h}	0.567 ± 0.008^{j}	87.20 ± 0.71^{ab}
	1.80	3.40 ± 0.80^{lm}	3.27 ± 0.33^{jk}	1.05 ± 0.11^{hi}	0.533 ± 0.007^{j}	88.01 ± 1.58^{ab}
	1.90	3.96 ± 0.77^{kl}	3.63 ± 0.09^{j}	1.40 ± 0.06^{fgh}	0.517 ± 0.008^{j}	88.92 ± 1.16^{a}
	1.20	4.70 ± 0.13^{jk}	4.79 ± 0.10^{i}	1.57 ± 0.06^{fg}	1.135 ± 0.005^{g}	78.08 ± 1.40^{efg}
	1.40	4.59 ± 0.11^{k}	5.29 ± 0.02^{h}	1.61 ± 0.05^{ef}	1.091 ± 0.006^{g}	79.16 ± 0.90^{ef}
II	1.65	5.65 ± 0.22^{ij}	5.31 ± 0.01^{h}	1.81 ± 0.08^{ef}	0.929 ± 0.007^{h}	80.36 ± 1.05^{de}
	1.80	5.67 ± 0.02^{ij}	6.42 ± 0.43^{g}	2.01 ± 0.06^{e}	0.917 ± 0.001^{h}	81.75 ± 0.40^{cd}
	1.90	5.83 ± 0.04^{i}	5.73 ± 0.10^{h}	1.78 ± 0.09^{ef}	0.892 ± 0.007^{h}	82.22 ± 0.27^{cd}
	1.20	7.61 ± 0.50^{h}	7.11 ± 0.36^{f}	1.63 ± 0.02^{ef}	1.622 ± 0.013^{c}	64.05 ± 1.15^{l}
	1.40	8.59 ± 0.23^{g}	8.12 ± 0.24^{e}	2.01 ± 0.02^{e}	1.596 ± 0.13^{c}	73.71 ± 0.60^{ij}
III	1.65	8.92 ± 0.15^{fg}	8.06 ± 0.06^{e}	2.32 ± 0.01^{cd}	1.465 ± 0.034^{e}	75.16 ± 0.24^{hi}
	1.80	9.79 ± 0.09^{ef}	8.46 ± 0.03^{e}	2.52 ± 0.01^{abc}	1.400 ± 0.104^{f}	76.57 ± 1.75^{fgh}
	1.90	10.36 ± 0.15^{de}	8.95 ± 0.09^{d}	2.58 ± 0.11^{abc}	1.351 ± 0.02^{f}	78.70 ± 0.00^{fg}
	1.20	11.04 ± 0.05^{cd}	9.39 ± 0.31^{cd}	2.58 ± 0.27^{abc}	2.298 ± 0.008^{a}	62.66 ± 0.82^{l}
	1.40	13.58 ± 0.34^{a}	11.07 ± 0.15^{a}	2.66 ± 0.30^{abc}	1.760 ± 0.013^{b}	63.62 ± 1.76^{l}
IV	1.65	11.68 ± 0.36^{bc}	9.22 ± 0.02^{d}	3.00 ± 0.31^{a}	1.644 ± 0.053^{c}	70.88 ± 0.12^{k}
	1.80	12.40 ± 0.45^{b}	9.75 ± 0.38^{c}	2.81 ± 0.34^{ab}	1.532 ± 0.012^{d}	72.87 ± 1.07^{jk}
	1.90	11.52 ± 0.09^{bc}	10.43 ± 0.24^{b}	2.97 ± 0.47^{a}	1.468 ± 0.002^{e}	76.91 ± 0.50
Feed I	-	7.12 ± 1.65	7.95 ± 0.17	1.99 ± 0.11	1.973 ± 0.011	63.39 ± 0.41
Feed II	-	10.25 ± 1.31	7.97 ± 1.60	2.63 ± 0.15	2.500 ± 0.014	53.65 ± 1.31
Feed III	-	14.03 ± 1.99	11.88 ± 0.93	3.37 ± 0.04	3.205 ± 0.040	44.06 ± 0.57
Feed IV	-	18.47 ± 2.63	12.69 ± 0.88	3.55 ± 0.06	3.425 ± 0.021	36.27 ± 0.77

Table 2. Analysis of properties of permeate microfiltration process at different cross flow velocity and stevioside concentration of feed with transmembrane pressure at 1.65 bar

Tabel 2. Analisis karakteristik permeat proses mikrofiltrasi pada berbagai kecepatan alir dan konsentrasi steviosida umpan dengan tekanan transmembran 1,65 bar

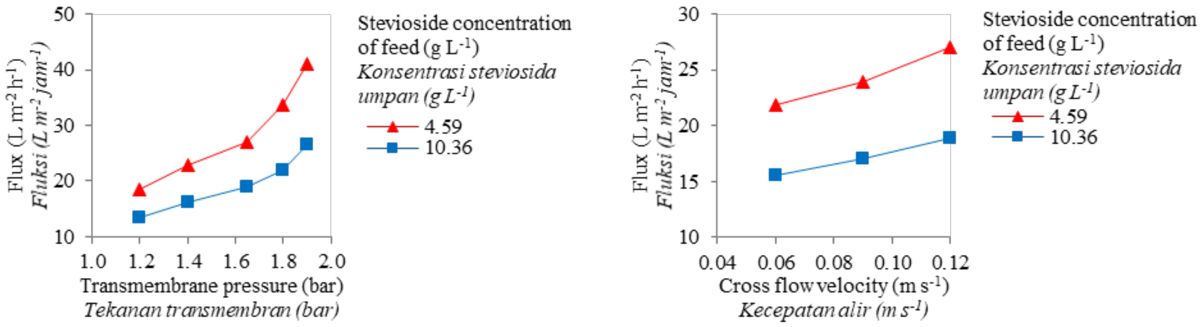
Feed concentration *Konsentrasi umpan*	Transmembrane pressure *Tekanan transmembran* (bar)	Stevioside *Steviosida* (g L^{-1})	Tannin *Tanin* (g L^{-1})	Total sugar *Total gula* (g L^{-1})	Color *Warna* (A)	Clarity *Kejernihan* (%T)
	0.04	4.02 ± 0.89^{e}	3.63 ± 0.16^{i}	1.36 ± 0.11^{e}	0.629 ± 0.008^{g}	86.90 ± 0.28^{a}
I	0.06	3.54 ± 0.82^{e}	3.35 ± 0.03^{j}	1.41 ± 0.09^{e}	0.623 ± 0.008^{g}	87.00 ± 0.71^{a}
	0.11	3.41 ± 0.84^{e}	3.64 ± 0.06^{i}	1.36 ± 0.01^{e}	0.567 ± 0.008^{h}	87.20 ± 0.71^{a}
	0.04	5.19 ± 0.30^{d}	5.67 ± 0.11^{g}	1.83 ± 0.08^{d}	1.027 ± 0.004^{e}	79.90 ± 1.69^{b}
II	0.06	6.69 ± 0.08^{c}	5.91 ± 0.06^{f}	1.88 ± 0.10^{d}	1.005 ± 0.004^{e}	80.17 ± 1.04^{b}
	0.11	5.65 ± 0.22^{cd}	5.31 ± 0.01^{h}	1.81 ± 0.08^{d}	0.929 ± 0.007^{f}	80.36 ± 1.05^{b}
	0.04	8.48 ± 0.23^{b}	8.50 ± 0.05^{d}	2.39 ± 0.00^{c}	1.592 ± 0.008^{c}	73.71 ± 1.32^{c}
III	0.06	9.43 ± 0.04^{b}	8.86 ± 0.03^{c}	2.47 ± 0.04^{c}	1.501 ± 0.018^{d}	75.08 ± 0.61^{c}
	0.11	8.92 ± 0.15^{b}	8.06 ± 0.06^{e}	2.32 ± 0.01^{c}	1.465 ± 0.034^{d}	75.16 ± 0.24^{c}
	0.04	11.59 ± 0.41^{a}	9.47 ± 0.00^{a}	2.64 ± 0.32^{bc}	1.707 ± 0.006^{a}	68.81 ± 2.58^{d}
IV	0.06	10.68 ± 0.30^{a}	9.25 ± 0.11^{b}	2.93 ± 0.10^{ab}	1.700 ± 0.009^{a}	69.35 ± 1.13^{d}
	0.11	11.68 ± 0.36^{a}	9.22 ± 0.02^{b}	3.00 ± 0.31^{a}	1.644 ± 0.053^{b}	70.88 ± 0.12^{d}
Feed I	-	7.12 ± 1.65	7.95 ± 0.17	1.99 ± 0.11	1.973 ± 0.011	63.39 ± 0.41
II	-	10.25 ± 1.31	7.97 ± 1.60	2.63 ± 0.15	2.500 ± 0.014	53.65 ± 1.31
III	-	14.03 ± 1.99	11.88 ± 0.93	3.37 ± 0.04	3.205 ± 0.040	44.06 ± 0.57
IV	-	18.47 ± 2.63	12.69 ± 0.88	3.55 ± 0.06	3.425 ± 0.021	36.27 ± 0.77

Cross flow ultrafiltration

The second step of the stevia extract purification process was carried out using ultrafiltration membrane. The comparison permeate and selected permeate produced by the microfiltration process were used as feed for the ultrafiltration process. The comparison permeate was obtained using feed with stevioside concentration of 10.25 g L^{-1} on transmembrane pressure at 1.40 bar and cross flow velocity at 0.11 m s^{-1}, the selected permeate was obtained using feed with stevioside concentration of 14.03 g L^{-1} at a transmembrane pressure of 1.90 bar and a cross flow velocity of 0.11 m s^{-1}. The decrease in permeate flux is phenomenon that often occurs in every membrane process, but its severity can be minimized by finding the correct transmembrane pressure and cross flow velocity. Appropriate transmembrane pressure allows the permeate to pass through the membrane at steady state, pressure difference between the feed side and the permeate can be applied to allow smaller molecules to pass through the semipermeable membrane from the feed stream to the permeate side, well as retaining larger molecules. Cross flow velocity can reduce the occurrence of fouling and concentration polarization, besides the right feed speed can help achieve better mass transport.

The graph of the relationship between the operating conditions of the ultrafiltration process and the permeate flux can be seen in Figure 5. The results show that the higher the transmembrane pressure and cross flow velocity, higher the permeate flux, while higher the feed concentration, lower the permeate flux. The resulting permeate flux phenomenon is the same as the permeate flux obtained during the microfiltration process. The resulting permeate flux is also the same as the study results (D´ıaz-Montes et al., 2020) increase in permeate flux can depend on increase in TMP, but after limiting TMP is reached, Jp does not increase significantly even though the operating pressure is increased. The highest permeate flux was obtained using feed with stevioside concentration of 4.59 g L^{-1} on transmembrane pressure at 1.90 bar. According to (Chhaya et al., 2012), permeate flux can increase with higher cross flow velocity at constant transmembrane pressure, higher cross flow rate causes convective flow cutting action, which limits the formation of cake layers so that resistance decreases and permeate flux increases. The shear force generated by cross flow can quickly remove foulant deposits on the membrane (Kirschner et al., 2019). The highest permeate flux was obtained using feed with stevioside concentration of 4.59 g L^{-1} on a cross flow velocity of 0.12 m s^{-1}.

Stevioside and tannin rejection rates in the ultrafiltration process tended to fluctuate with the increase in transmembrane pressure and cross flow velocity, but the resulting rejection rate was affected by the feed concentration. The results of the permeate characteristic analysis showed that the ultrafiltration membrane could reject stevioside and tannin. Stevioside rejection rates ranged from 17.54 % - 49.15 %, and tannin rejection rates ranged from 39.05 % - 48.95 %. Dynamic cake layer can withstand high molecular weight components at high operating pressure, thereby increasing the clarity of the permeate, but the irreversible fouling that occurs can reduce the concentration of stevioside in the permeate (Chhaya et al., 2012). MWCO has an essential role in the separation process which can reject solutes, then the intrinsic properties of the membrane, such as the type of material, surface topography, and hydrophilicity, lead to chemical interactions between the membrane and compounds resulting in fouling (Castro-Muñoz et al., 2021).



(a) (b)

Figure 5. Graph of the relationship between (a) transmembrane pressure to permeate flux at two feed concentrations with cross flow velocity at 0.12 m s^{-1} and (b) cross flow velocity to permeate flux at two stevioside concentrations of feed with transmembrane pressure at 1.65 bar

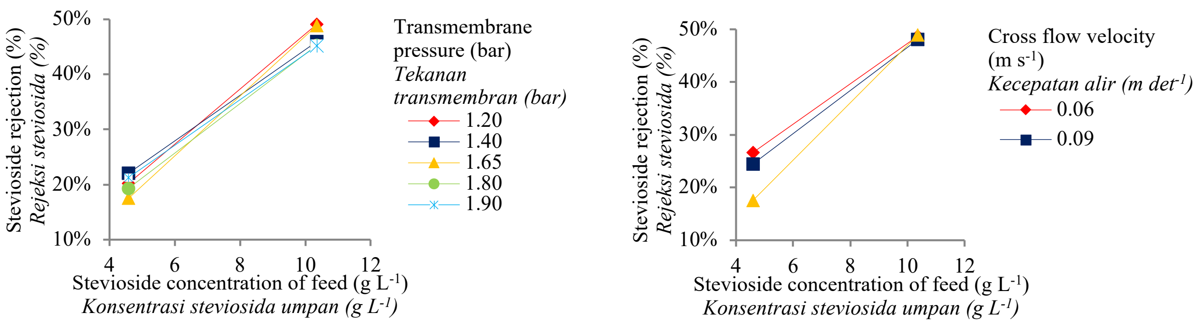
Gambar 5. Grafik hubungan antara (a) tekanan transmembran terhadap fluksi permeat pada dua konsentrasi umpan dengan kecepatan alir 0,12 m det^{-1} dan (b) kecepatan alir terhadap fluksi permeat pada dua konsentrasi steviosida umpan dengan tekanan transmembran 1,65 bar

The total sugar content produced was also relatively fluctuated with increase in transmembrane pressure and cross flow velocity, but the concentration of feed with stevioside concentration of 4.59 g L^{-1} or comparison feed produced the highest total sugar content. Increasing transmembrane pressure and cross flow velocity can decrease color and increase clarity, but higher feed concentrations can result in stronger color and lower clarity of permeate. The lowest stevioside rejection of 17.54 % was obtained using comparison feed on a transmembrane pressure at 1.65 bar and a cross flow velocity at 0.12 m s^{-1} with tannin rejection of 43.18 %, total sugar content of 66.90 %, color reduction of 54.64 %, and clarity of 91.31 %. The microfiltration process used feed with stevioside concentration of 10.25 g L^{-1} at transmembrane pressure of 1.40 bar and a cross flow velocity of 0.11 m s^{-1}, followed by ultrafiltration process at a transmembrane pressure of 1.65 bar and a cross flow velocity at 0.12 m s^{-1} resulting in total stevioside rejection of 63.12 % and total rejection of tannins of 62.27 %. The ultrafiltration process using the selected feed (the best permeate from microfiltration process) can produce the lowest stevioside rejection of 45.17 % on transmembrane pressure at 1.90 bar and cross flow velocity at 0.12 m s^{-1} with permeate flux of 26.51 L m^{-2} h^{-1}. This best operating condition can reject tannins by 44.24 % with total sugar content of 48.37 %, decrease in the color of 55.04 %, and clarity of 86.80 % T.

The results of analyzing the properties of the permeate ultrafiltration process at different transmembrane pressures and feed concentrations with a cross flow velocity at 0.12 m s^{-1} are shown in Table 3. The properties of the permeate ultrafiltration process at different cross flow velocities and feed concentrations with transmembrane pressure at 1.65 bar can be seen in Table 4. Analysis of variance showed that the interaction between the stevioside concentration of feed (4.59 and 10.36 g L^{-1}) with transmembrane pressure (1.20, 1.40, 1.65, 1.80, and 1.90 bar) and cross flow velocity (0.06, 0.09, and 0.12 m s^{-1}) significantly affected the characteristics of permeate ultrafiltration processes, such as stevioside, tannin, total sugar, color, and clarity. The ultrafiltration process reduced dramatically the tannin content in the permeate.

The best operating conditions of the microfiltration and ultrafiltration processes resulted in total stevioside rejection of 59.52 % and total tannin rejection of 57.99 %. Overall, the stevia extract purification process using microfiltration and ultrafiltration membranes was able to remove total solids and non-steviol glycoside compounds, but the rejection rate for stevioside was still relatively high. The type of PVC polymer membrane used has hydrophobic properties that are prone to fouling which results in the adsorption of more solutes or macromolecular particles to the surface and pores of the membrane, which can result in a decrease in permeate flux over time of operation, but based on the specifications, the microfiltration and ultrafiltration membranes used have been modified hydrophilic. On the other hand, the pore size of the membrane has an essential role in stevioside rejection because the high molecular weight component contained in the stevia extract can form dynamic cake layer on the surface of the membrane.

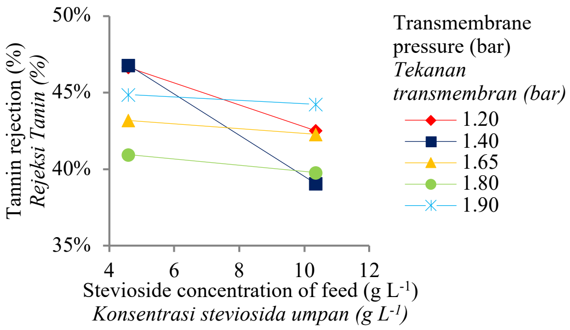
The microfiltration process is prone to fouling due to its larger pore size so that dissolved particles can settle in the pores and partially or completely block them (Chhaya et al., 2013). According to Das et al. (2015), ultrafiltration membranes with 30 kDa MWCO could produce higher concentrations of rebaudioside A and stevioside than ultrafiltration membranes with 10, 20, and 50 kDa MWCO. Ultrafiltration membranes with 30 kDa MWCO can retain high concentrations of steviol glycoside compounds because the fouling phenomenon is more controlled, and the hydrodynamic properties are more compatible with the components contained in stevia extract (Castro-Muñoz et al., 2021).



(a) (b)

Figure 6. Graph of the relationship between (a) transmembrane pressure (TMP) and (b) cross flow velocity (CFV) on stevioside rejection in the ultrafiltration process

Gambar 6. Grafik hubungan antara (a) tekanan transmembran (TMP) dan (b) kecepatan alir (CFV) terhadap rejeksi steviosida pada proses ultrafiltrasi



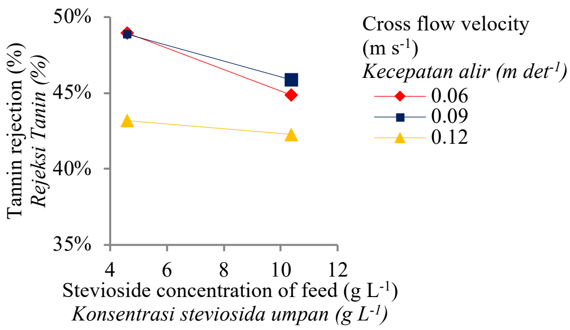


Figure 7. Graph of the relationship between (a) transmembrane pressure (TMP) and (b) cross flow velocity (CFV) on tannin rejection in the ultrafiltration process

Gambar 7. Grafik hubungan antara (a) tekanan transmembran (TMP) dan (b) kecepatan alir (CFV) terhadap rejeksi tanin pada proses ultrafiltrasi

Table 3. Analysis properties of permeate ultrafiltration process at different transmembrane pressures and stevioside concentrations of feed with cross flow velocity of 0.12 m s^{-1}

Tabel 3. Analisis karakteristik permeat proses ultrafiltrasi pada berbagai tekanan 45ransmembrane dan konsentrasi steviosida umpan dengan kecepatan alir 0,12 m det^{-1}

Feed concentration *Konsentrasi umpan*		Transmembrane pressure *Tekanan 45ransmembrane* (bar)	Stevioside *Steviosida* (g L^{-1})	Tannin *Tanin* (g L^{-1})	Total sugar *Gula total* (g L^{-1})	Color *Warna* (A)	Clarity *Kejernihan* (%T)
		1.20	3.66 ± 0.09^{c}	2.82 ± 0.05^{b}	0.82 ± 0.03^{c}	0.44 ± 0.006^{d}	84.63 ± 0.41^{d}
		1.40	3.58 ± 0.04^{c}	2.82 ± 0.06^{b}	0.93 ± 0.11^{bc}	0.42 ± 0.005^{e}	89.54 ± 0.29^{b}
I		1.65	3.78 ± 0.25^{c}	3.01 ± 0.07^{b}	1.08 ± 0.26^{abc}	0.42 ± 0.006^{ef}	91.31 ± 0.15^{a}
		1.80	3.70 ± 0.03^{c}	3.13 ± 0.53^{b}	0.82 ± 0.01^{c}	0.41 ± 0.008^{ef}	91.41 ± 0.30^{a}
		1.90	3.61 ± 0.11^{c}	2.92 ± 0.12^{b}	0.86 ± 0.03^{c}	0.41 ± 0.009^{f}	91.83 ± 0.00^{a}
		1.20	5.27 ± 0.02^{b}	5.15 ± 0.05^{a}	1.22 ± 0.10^{ab}	0.76 ± 0.004^{a}	81.85 ± 0.27^{e}
		1.40	5.60 ± 0.22^{ab}	5.45 ± 0.08^{a}	1.21 ± 0.11^{ab}	0.76 ± 0.006^{a}	84.82 ± 1.24^{d}
II		1.65	5.30 ± 0.06^{b}	5.17 ± 0.07^{a}	1.21 ± 0.15^{ab}	0.73 ± 0.005^{b}	86.60 ± 0.70^{c}
		1.80	5.66 ± 0.18^{a}	5.39 ± 0.54^{a}	1.26 ± 0.10^{a}	0.73 ± 0.011^{bc}	86.70 ± 0.85^{c}
		1.90	5.68 ± 0.24^{a}	4.99 ± 0.02^{a}	1.25 ± 0.10^{a}	0.72 ± 0.009^{c}	86.80 ± 0.42^{c}
Feed or permeat MF	I	1.40 (II, 0.11 m s^{-1})	4.59 ± 0.11	5.29 ± 0.02	1.61 ± 0.05	1.091 ± 0.006	79.16 ± 0.90
	II	1.90 (III, 0.11 m s^{-1})	10.36 ± 0.15	8.95 ± 0.09	2.58 ± 0.11	1.351 ± 0.02	76.91 ± 0.50

Table 4. Analysis properties of permeate ultrafiltration process at different cross flow velocity and stevioside concentration of feed with transmembrane pressure at 1.65 bar

Tabel 4. Analisis karakteristik permeat proses ultrafiltrasi pada berbagai kecepatan alir dan konsentrasi steviosida umpan dengan tekanan 45ransmembrane 1,65 bar

Feed concentration *Konsentrasi umpan*		Transmembrane pressure *Tekanan 45ransmembrane* (bar)	Stevioside *Steviosida* (g L^{-1})	Tannin *Tanin* (g L^{-1})	Total sugar *Gula total* (g L^{-1})	Color *Warna* (A)	Clarity *Kejernihan* (%T)
		0.06	3.37 ± 0.00^{c}	2.70 ± 0.01^{d}	0.82 ± 0.03^{b}	0.42 ± 0.005^{c}	90.26 ± 0.73^{a}
I		0.09	3.47 ± 0.04^{bc}	2.71 ± 0.02^{d}	0.86 ± 0.00^{b}	0.42 ± 0.007^{c}	90.78 ± 0.59^{a}
		0.12	3.78 ± 0.25^{b}	3.01 ± 0.07^{c}	1.08 ± 0.26^{ab}	0.42 ± 0.006^{c}	91.31 ± 0.15^{a}
		0.06	5.32 ± 0.21^{a}	4.93 ± 0.01^{b}	1.26 ± 0.06^{a}	0.76 ± 0.003^{a}	85.80 ± 0.70^{b}
II		0.09	5.38 ± 0.14^{a}	4.84 ± 0.02^{b}	1.31 ± 0.03^{a}	0.75 ± 0.006^{a}	85.90 ± 0.56^{b}
		0.12	5.30 ± 0.06^{a}	5.17 ± 0.07^{a}	1.21 ± 0.15^{a}	0.73 ± 0.005^{b}	86.60 ± 0.70^{b}
Feed (permeat UF)	I	1.40 (C2 dan 0.11 m s^{-1})	4.59 ± 0.11	5.29 ± 0.02	1.61 ± 0.05	1.091 ± 0.006	79.16 ± 0.90
	II	1.90 (C3 dan 0.11 m s^{-1})	10.36 ± 0.15	8.95 ± 0.09	2.58 ± 0.11	1.351 ± 0.02	76.91 ± 0.50

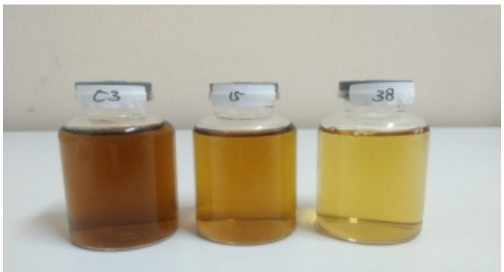


Figure 8. Clarity of stevia extract (left), microfiltration of permeate (center), and microfiltration + ultrafiltration of permeate (right)

Gambar 8. Kejernihan ekstrak stevia (kiri), permeat mikrofiltrasi (tengah) dan permeat mikrofiltrasi + ultrafiltrasi (kanan)

Conclusion

The best operating conditions for the microfiltration process were using stevioside concentration feed of 14.03 g L^{-1} at a transmembrane pressure of 1.90 bar and a cross flow velocity of 0.11 m s^{-1} with a permeate flux of 82.90 L m^{-2} h^{-1}. Best operating conditions for the ultrafiltration process were using the selected feed at a transmembrane pressure of 1.90 bar and a cross flow velocity of 0.12 m s^{-1} with a permeate flux of 26.51 L m^{-2} h^{-1}. The best operating conditions of the microfiltration and ultrafiltration processes resulted in 59.52 % total stevioside rejection and 57.99 % total tannin rejection.

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