

Ultrafiltration for the separation of polyphenol oxidase and peroxidase and its effect on physicochemical and antioxidant properties of coconut (*Cocos nucifera* L.) water

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Abstract

Coconut water is a nutritious, natural, and refreshing drink. However, a challenge for the coconut water industry is its fast deterioration caused by naturally occurring enzymes mainly polyphenol oxidase (PPO) and peroxidase (POD). This study aimed to separate the PPO and POD using ultrafiltration and to evaluate its effect on the physicochemical properties and antioxidant attributes of tender and mature coconut water. The membrane used was polyethersulfone (PES) with two molecular weight cut-offs, namely 30 kDa (PES 30) and 50 kDa (PES 50) and conducted in a vacuum filter unit. The results showed that ultrafiltration with PES 30 completely separated the POD activity of both tender and mature coconut water. The ultrafiltration was also able to separate 86.07% PPO activity of tender coconut water and 100% separation on mature coconut water. While PES 50 could separate 49.60% PPO activity in tender coconut water. The ultrafiltration process also increased the clarity of mature coconut water and maintained the total sugar, sodium, and potassium contents in both maturities. However, ultrafiltration using these membrane types reduced total phenolic content and radical scavenging activity. In conclusion, this study revealed that ultrafiltration can remove the oxidative enzymes to extend the shelf life against oxidation deterioration. Further studies should be conducted to investigate the separation of enzymes without high significance loss of polyphenols, antioxidants, and nutritional properties.

1. Introduction

Coconut water is the juice that is obtained from coconut fruit aged 6 months and over. This liquid contains nutrients such as glucose, fructose, sucrose, malic acid (Sucupira *et al.*, 2017), vitamin B1, B3, B5 (Cappelletti *et al.*, 2015), potassium, sodium, calcium, magnesium, phosphor, zinc, iron (Santoso *et al.*, 1996a; Kwiatkowski *et al.*, 2008), as well as protein and fat in small amounts (Purkayastha *et al.*, 2012; Tan *et al.*, 2014). Besides, coconut water also contains a wide range of polyphenols substances that have antioxidant potentials such as salicylic acid, catechins, and epicatechins. A small amount of hydroxybenzoic acid, syringic acid, p-coumaric acid, coumaric acid, and caffeic acid was also detected in the coconut water (Chang and Wu, 2011; Mahayothee *et al.*, 2016). Antioxidative effect of coconut water extract on TBARS (Thiobarbituric Acid Reactive Substances) value in the liver of rats fed fish oil diet has been reported by Santoso *et al.* (1996b).

Commonly, the water consumed is that from the tender coconut due to its better taste and appearance. Whereas mature coconut water also has some good traits such as higher antioxidant activity, polyphenols content (Appaiah *et al.*, 2015), and minerals content (Tan *et al.*, 2014). Some process has been developed to increase the acceptability of mature coconut water such as the addition of lemon juice (Chauhan *et al.*, 2012) and fermentation (Zhang *et al.*, 2018) has been done. Another process to increase its value in the food industry is expected.

A challenge for the food industry is that coconut water undergoes fast deterioration. Although the liquid is sterile in its endocarp, it will undergo some physicochemical changes during the process until the product reaches the consumer. This change is caused by the activity of naturally occurred enzymes such as polyphenol oxidase (PPO) and peroxidase (POD) (Thaisakornphun and Tongchitpakdee, 2018) as well as contamination by microorganisms from the environment

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when processed under non-aseptic conditions (Awua *et al.*, 2012; Maciel *et al.*, 2013). Piló *et al.* (2009) reported that there was no microorganism found in coconut water treated aseptically.

The presence of PPO and POD in coconut water caused the water to turn pink during storage (Garcia *et al.*, 2007). Besides, PPO can catalyze the hydroxylation of monophenol and the oxidation of o-hydroxy phenol (Vigyázó and Haard 1981). Meanwhile, peroxidase can oxidize phenolic and non-phenolic compounds (Nokthai *et al.*, 2010). The processing technology is expected to eliminate the enzymes and improve appearance while maintaining some of its chemical and antioxidant properties. Heat treatment is the most common method used to inactivate enzymes (Murasaki-Aliberti *et al.*, 2009). However, the use of heat caused the decrease of brightness (Cappelletti *et al.*, 2015) and forming a "cooked" aroma (De Marchi *et al.*, 2015).

Filtration is a processing technology without the use of heat that can be applied to liquid raw materials. The principle of this technology is to separate solids from solutions based on their size and molecular weight. This process is regarded as safe, easy, energy-efficient, and environmentally friendly. Some of the processing of tender coconut water using filtration process has been carried out using stratified filtering, microfiltration, and ultrafiltration. The stratified filtering and microfiltration applied to coconut water can remove bacteria, yeast, and fungi (Reddy *et al.*, 2007) but still show enzyme activity (Purkayastha *et al.*, 2012). Meanwhile, ultrafiltration can maintain total soluble solids, acidity, density, pH (Jayanti *et al.*, 2010) and minerals (Laorko *et al.*, 2008) and can reduce enzyme activity on the use of 20 kDa and 30 kDa membranes (Nakano *et al.*, 2011; Debien *et al.*, 2013). The leakage can be caused by the large pore size on the microfiltration membrane that has not been able to hold the PPO and POD enzymes, which have a molecular weight of 64.8 kDa (Demir *et al.*, 2012) and 44.63 kDa (Duarte *et al.*, 2002), consecutively.

Polyethersulfone membrane is a hydrophilic membrane with a wide pH range and operating temperature for various food processing. It is widely used in the processing of apple juice (Borneman *et al.*, 1997; Susanto *et al.*, 2020), pineapple wine (Youravong *et al.*, 2017), broccoli juice (Yilmaz and Bagci, 2019), and sugarcane juice (Saha *et al.*, 2009). The polyethersulfone membrane can separate protein and polyphenol oxidase from potato fruit juice (Schmidt *et al.* 2016). The molecular weight cut off (MWCO) of 30 kDa and 50 kDa were selected according to PPO and POD molecular weight. The ultrafiltration process in coconut water is generally carried out on tender coconut water and has never been applied to mature coconut

water treatment. At the same time, ultrafiltration is expected to improve the appearance of mature coconut water and its utility in the food industry. Besides, the ultrafiltration process of tender and mature coconut water using 50 kDa PES membranes has never been carried out, and its effect on antioxidant properties is still scarcely studied. So it is necessary to study the use of PES membranes with molecular weights of 30 kDa and 50 kDa in the processing of both tender and mature coconut water. Ultrafiltration using polyethersulfone is expected to eliminate POD and PPO enzymes while retaining the nutrition and improving the physical properties of coconut water.

The objectives of this study are to determine the effect of the ultrafiltration using polyethersulfone with two different molecular weight cut-offs (30 kDa and 50 kDa) on the removal of polyphenol oxidase and peroxidase enzymes and its effect on the physicochemical properties, total phenolic compound, and antioxidant activity properties of both tender and mature coconut water.

2. Materials and methods

2.1 Raw materials and reagents

Coconut water (*Cocos nucifera* L.) used was tender and mature coconut fruits of Tall variety obtained from Bantul, Yogyakarta, Indonesia. The tender coconut fruits were 7 months old and contained a thin coconut flesh that looks like jelly. The mature coconut fruits were 11 months old and contained a hard thick layer of coconut flesh. Coconut fruits were randomly selected and cleaned using running water to remove the plant debris. The tools in contact with the fruits were cleaned with running water and boiled in boiling water before using them. The coconuts were placed on a cutting board and cut using a stainless steel knife. Coconut water was then filtered using a stainless steel filter to remove the remaining coir and was collected in a beaker glass for volume measurement. The water was put in a sterile falcon container and stored at -18°C until analysis.

The reagents used were 70% alcohol, HCl, Pb acetate, Na oxalate, NaOH, Na₂CO₃, KNaC₄H₄O₆·4H₂O, NaHCO₃, Na₂SO₄, CuSO₄·5H₂O, (NH₄)₂MoO₄, H₁₅Na₂AsO₁₁, Folin-Ciocalteu reagent, DPPH, D-glucose, quercetin, ascorbic acid, catechins, phosphate buffers, guaiacol (Himedia), hydrogen peroxide, pyrocatechol, nutrient agar, standard potassium and sodium, CaCO₃, H₂SO₄, methanol, ethanol, and phenol. All chemicals come from Merck company, USA unless specified.

2.2 Membranes

The membranes that were used in this study are Polyethersulfone 30 kDa (PES 30) and Polyethersulfone 50 kDa (PES 50) (Synder Filtration, USA). The membrane preparation was carried out by flowing 100 mL of non-ionic water on the membrane attached to the ultrafiltration device to remove the remaining organic material. Membrane replacement was carried out in each experiment.

2.3 Filtration unit and membrane preparation

Foam and stainless steel plates were washed and boiled in boiling water before use, while ultrafiltration tools such as feed and permeate containers were washed, rinsed, and poured by boiling water. Furthermore, the ultrafiltration tool was allowed to cool before use. This treatment was carried out before and after each experiment. The design of the vacuum filtration unit is shown in Figure 1.

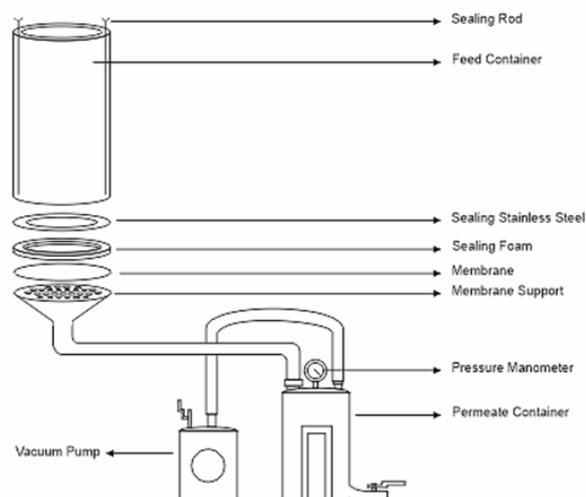


Figure 1. Ultrafiltration design with a vacuum pump

2.4 Ultrafiltration process

An amount of 200 mL of tender or mature coconut water was poured into a vacuum filtration unit installed with an ultrafiltration membrane with a surface area of 132,665 cm². The ultrafiltration tool model used was a vacuum filter with a dead-end system. The membrane was placed on top of the membrane support and held using foam and stainless steel plates to ensure a vacuum condition in the permeate container. The pressure on the vacuum pump (Value VE 280 N) was set at -70 cmHg before the process is carried out. Coconut water in a permeate container was then stored in sterilized falcon tubes and stored in a refrigerator at -18°C until analysis. The analysis carried out included permeate flux, transmittance, total sugar, potassium, sodium, antioxidant activity, total phenolic content, PPO, and POD enzyme activity. While the membranes used were

analyzed using FTIR.

2.5 Thermal process

The thermal process treatment was carried out based on the method of Thaisakornphun and Tongchitpakdee (2018). Pasteurization was carried out using 200 mL of coconut water heated at 90°C for 10 mins. Hereafter, the coconut water sample was cooled by immersing the pasteurization container containing coconut water in cold water. The thermal process in this study was carried out as a comparison to fresh and ultrafiltered coconut water.

2.6 Analysis

2.6.1 Flux permeate

The flux permeate analysis was carried out based on Debien *et al.* (2013). The permeate flux (J) is the mass that passes through the membrane per unit area per time, M_p (kg) is the permeate mass, t is the time (h), and A_p is the permeate area (m²). The following formula calculates the permeate flux:

$$J = \frac{M_p}{A_p \cdot t}$$

2.6.2 Transmittance

The analysis was carried out based on Tan *et al.* (2014). The transmittance assay was conducted to measure the clarity of coconut water. The transmittance of coconut water and distilled water was measured at 610 nm on spectrophotometer Thermo-scientific Genesys 10S UV-Vis.

2.6.3 Density

The pycnometer was previously calibrated using distilled water with a temperature of 27°C. The dry and clean pycnometer was weighed on an analytical scale. Then the distilled water was inserted into the pycnometer carefully to prevent bubbles in the instrument. The appliance was closed and dried using a tissue. After that, the pycnometer that has been filled with distilled water was weighed, and the volume was calculated using the formula:

$$\text{Volume} = \frac{\text{End pycnometer weight} - \text{initial pycnometer weight}}{0.996512}$$

Where 0.996512 was the specific gravity of water at a temperature of 27°C. The volume listed after the calibration was then used to measure the density of the sample. The measurement of sample density was carried out in the same manner as the calibration procedure and was calculated using the following formula:

$$\rho \text{ (g/cm}^3\text{)} = \frac{\text{End pycnometer weight} - \text{initial pycnometer weight}}{\text{volume}}$$

2.6.4 Total sugar

Total sugars were analyzed according to AOAC

(2005) by the Nelson-Somogyi method with a modification. One mL of coconut water was poured into a beaker glass, and 50 mL of distilled water was added. Ten drops of 40% Pb acetate were added to the solution, and ten drops of Na oxalate was added to precipitate Pb acetate. Aquadest was added to the limit of 100 mL. Then homogenized and filtered using filter paper. A 60 mL of filtrate was poured in a 250 mL Erlenmeyer flask, and 5 mL of 30% HCl was added. The filtrate was then heated in a water bath at 70°C for 20 mins then cooled down with running water. The cooled filtrate was then added with 40% NaOH until the pH returned to neutral, then aquadest was added to 100 mL and homogenized. A 1 mL of the final filtrate was poured into a test tube and then added 1 mL of nelson A:B reagents (25:1). The mixture was then heated at a water temperature of 70°C for 20 mins. The test tube was cooled with running water, reacted with 1 mL of arsenomolybdate and homogenized. A volume of 7 mL distilled water was added into the test tube, homogenized, and the absorbance was read at a wavelength of 540 nm. The stock solution of glucose was prepared at 1 mg/mL. The dilution was done to give a final concentration of 0, 0.2, 0.4, 0.6, 0.8, 1.0 in aquadest. All the standard solution was treated as those of coconut water. The percentage of total sugar is calculated with the following formula:

$$\text{Total sugar (\%)} = \frac{\text{The concentration of sample} \times \text{dilution factor}}{\text{weight of sample (mg)}} \times 100\%$$

2.6.5 Sodium and potassium

Sodium and potassium content was determined using Atomic Absorption Spectroscopy (AAS) based on the method of Apriyantono *et al.* (1989). Ash solution was made by wet ashing using dilute acid, and the ashes were transferred into a measuring flask and diluted to the limit of detection and the operating range. Potassium was read at 766.5 Å, while sodium was read at a wavelength of 589.0 Å. Both minerals were read at a metal detection limit of 0.002 µg metal/mL and a working range of 0.1 - 5 µg metal/mL. Furthermore, samples, standard solutions, and blanks were put into the AAS tool to analyze absorption at each metal wavelength. During sample measurements, periodic checks need to be carried out to see whether the standard value remains constant. Next, a standard curve was made for each metal (absorbance value vs. metal concentration in µg metal/mL).

2.6.6 Total phenolic content (TPC)

The analysis was done following Mahayothee *et al.* (2016) using Folin Ciocalteu's reagent. A 0.2 mL coconut water was mixed with 1 mL of 10% v/v Folin Ciocalteu's reagent and allowed to react for 3 mins. 0.8 mL of 7.5% w/v sodium carbonate was added to the

mixture and incubated for two h at room temperature. The absorbance of the mixture was then measured at 765 nm using a spectrophotometer.

The stock solution of gallic acid was prepared at 100 mg/L, and the dilution was done to give a final concentration of 0, 20, 40, 60, 80, 100 mg/L in aquadest. All the standard solution was treated as those of coconut water. The phenolic content was calculated with the following formula:

$$\text{TPC (mg (GAE)/100 mL)} = \frac{\text{The concentration of sample} \times \text{dilution factor} \times \text{total volume of sample}}{\text{the volume of sample} \times 10}$$

2.6.7 Radical scavenging activity (RSA)

The radical scavenging activity assay was done using the DPPH method based on Brand-Williams *et al.* (1995) with modification. A 0.1 mM stock solution of DPPH was prepared in methanol. A 3.5 mL of DPPH solution was added into 0.5 mL coconut water and homogenated using a vortex. The solution was allowed to react for 30 mins and read at the wavelength of 515 nm while 0.5 mL methanol and 3.5 mL DPPH were used as the blank. RSA was calculated using the following formula:

$$\text{DPPH Radical scavenging activity RSA \%} = \frac{\text{Blank absorbance} - \text{sample absorbance}}{\text{Blank absorbance}} \times 100$$

2.6.8 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR testing was carried out based on Kaewruang *et al.* (2014). A total of 2 mg of the dry membrane and 200 mg of potassium bromide (KBr) were mixed in a vibrating ball and molded using a screw press until a thin pellet was obtained. Furthermore, the pellets were placed in a tablet holder to be tested using an infrared IR Prestige-21 spectrophotometer using a high sensitivity DLATGS (Deuterated Lanthanum α -Alanine doped TriGlycine Sulphate) detector, which was measured at a wavelength of 400-4000 cm⁻¹.

2.6.9 Enzyme activity

The activity of polyphenol oxidase and peroxidase enzymes was tested based on Debien *et al.* (2013). Polyphenol oxidase activity testing was carried out using pyrocatechol as a substrate. 1.3 mL of 0.35 M phosphate buffer (pH 6.0), 0.7 mL of 0.2 M pyrocatechol, and 2 mL of sample were poured into a test tube. The mixture was then homogenized using a vortex, and the absorbance changes were read using spectrophotometer UV-Vis at 425 nm. Blank measurements were carried out using a buffer solution and pyrocatechol with the same concentration.

Peroxidase activity was tested using guaiacol as a substrate and H₂O₂ as a hydrogen donor. A 1.3 mL of 0.35 M phosphate buffer (pH 5.5) was poured into a test tube and heated in a water bath until the buffer

temperature reached 35°C. Subsequently 2 mL of sample, 0.2 mL of 0.1% H₂O₂, and 0.5 mL of 0.5% guaiacol was added. The mixture was then mixed using a vortex, and the absorbance was read using a UV-Vis spectrophotometer at 470 nm. Blank measurements were carried out using a buffer solution, guaiacol, and H₂O₂ with the same concentration.

The absorbance readings were carried out every 30 secs for 10 mins. One unit of enzyme is equivalent to a change of 0.001 absorbances per min per ml of sample. The enzyme activity is calculated using the formula:

$$\text{Activity (U/ml)} = \frac{(A_{\text{sample}} - A_{0 \text{ sample}}) - (A_{\text{blank}} - A_{0 \text{ blank}})}{0.001 \times t}$$

Where A = final absorbance, A₀ = Initial absorbance and t = time (min)

Enzyme activity retention is calculated using the formula:

$$R = 100 \times \left(1 - \frac{A_p}{A_A}\right)$$

R is the level of enzyme activity retention, A_A and A_p are the control and permeate enzyme activity, respectively.

2.7 Statistical analysis

A non-factorial ANOVA and Duncan test with 0.05 level of significance (P<0.05) were used to analyze the ultrafiltration data. IBM SPSS Statistics version 20 (IBM Corporation, New York, USA) was used to perform the statistical analysis.

3. Results and discussion

3.1 Permeate flux

One of the things that need to be considered in processing using ultrafiltration is the permeate flux. The permeate flux of tender and mature coconut water using PES 30 and 50 is shown in Figure 2. The graph in Figure 2 shows a sharp decrease in the first 10 mins of the filtration process on all membranes used. The filtration system used in this study was the dead-end type that allows the fluid to be pushed or pulled towards the membrane using pressure to ensure that molecules weighing greater than MWCO can accumulate on the membrane surface and causing rapid clogging. The fouling and blockages can be caused by polysaccharides, enzymes, and proteins in coconut water (Lamdande *et al.*, 2020).

The filtration using PES 50 had a lower permeate flux and longer processing time than PES 30. At the beginning of the filtration process, some molecules can pass through the membrane pores. Still, particles with a size similar to the membrane pores, such as the POD enzyme, have a molecular weight ranging from 44.63 -

49 kDa (Duarte *et al.*, 2002; Balasubramanian and Boopathy, 2013) can cause blockages in the pores. Whereas on the PES 30 membrane, the MWCO smaller than the enzyme molecules causes no blockage in the membrane pores to have a higher permeate flux. Moreover, Laorko *et al.* (2010) and Mohammad and Amin (2013) reported that membranes with larger MWCO are more prone to pore-clogging, reducing the membrane pore size. The lower permeate flux and longer processing time for tender coconut water processing were attributed to the higher total dissolved solids found in tender coconut water than mature coconut water.

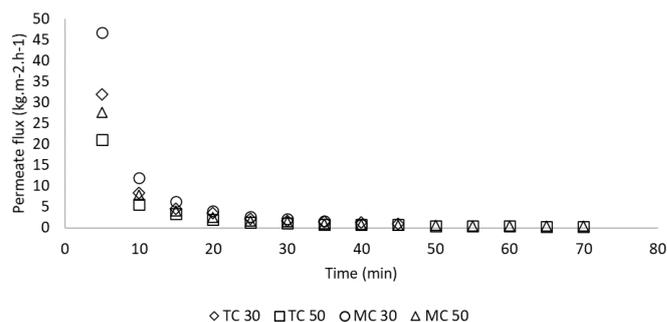


Figure 2. Permeate flux of ultrafiltration process in tender and mature coconut water using PES 30 and 50 membranes. TC30: tender coconut water treated with PES 30, TC50: tender coconut water treated with PES 50, MC30: mature coconut water treated with PES 30, MC50: mature coconut water treated with PES 50.

The decrease in permeate flux during the filtration process can be explained based on four mechanisms: complete blockage, common blockage, intermediate blockage, and a cake layer on the membrane surface (Salahi *et al.*, 2010). Based on this phenomenon, it is suspected that the blockage that occurs in PES 50 is an intermediate type of blockage. Intermediate blockage occurs when the particle size is similar to the pore size of the membrane. Besides, in this type of blockage, the particles are not entirely retained by the membrane (Salahi *et al.*, 2010). In the POD enzyme parameter, PES 50 still shows enzyme activity, although a little. This indicates the presence of enzyme molecules passing through the membrane during the initial filtration process.

3.2 Physical and chemical characteristics

The physical and chemical characteristics of tender and mature coconut water with or without treatment can be seen in Table 1. The ultrafiltration process showed a significant difference (P<0.05) between TCC, MC30, and MC50, on the transmittance parameter. There is also a significant difference (P<0.05) between the tender and mature coconut water groups in total sugar and sodium parameters. However, insignificant differences (P>0.05) were seen in the parameters density and potassium from

Table 1. Physical and chemical characteristics of control tender and mature coconut water, after filtration with PES 30, PES 50, and thermal process

Sample	Transmittance (%)	Density (g/mL)	Total sugar (%)	Potassium (mg/100 mL)	Sodium (mg/100 mL)
TCC	92.31±7.68 ^{ab}	1.020±0.00 ^a	13.54±1.95 ^a	139.86±41.47 ^a	7.40±6.46 ^b
TC30	94.68±7.12 ^a	1.020±0.00 ^a	10.84±1.99 ^{ab}	103.17±54.52 ^a	5.74±1.78 ^b
TC50	95.09±5.40 ^a	1.022±0.00 ^a	12.12±0.30 ^{ab}	116.64±33.98 ^a	6.90±1.41 ^b
TCT	87.42±8.26 ^{abc}	1.020±0.00 ^a	13.95±3.25 ^a	139.88±0.63 ^a	6.96±3.46 ^b
MCC	81.93±7.36 ^{bc}	1.018±0.00 ^a	8.26±2.60 ^c	116.64±23.52 ^a	34.18±9.90 ^a
MC30	96.57±3.32 ^a	1.018±0.00 ^a	7.60±2.47 ^c	96.83±0.67 ^a	26.22±0.82 ^a
MC50	95.83±3.06 ^a	1.019±0.00 ^a	8.33±2.90 ^c	131.69±53.02 ^a	30.36±13.13 ^a
MCT	79.64±8.82 ^c	1.021±0.00 ^a	9.62±2.27 ^{cb}	146.74±66.09 ^a	33.88±10.33 ^a

TCC: untreated tender coconut water, TC30: tender coconut water treated with PES 30, TC50: tender coconut water treated with PES 50, TCT: tender coconut water heated at 90°C for 10 mins, MCC: untreated mature coconut water, MC30: mature coconut water treated with PES 30, MC50: mature coconut water treated with PES 50, MCT: mature coconut water heated at 90°C for 10 mins. Values are expressed as mean±SD. Values with different superscript in the same column are significantly different ($P<0.05$) between samples.

all treatments. The table shows that the ultrafiltration process using PES 30 and PES 50 membranes can improve physical properties while maintaining several chemical properties in coconut water.

A transmittance assay was conducted to measure the clarity of coconut water. The higher the transmittance, the higher the clarity. Mature coconut water that went through an ultrafiltration process using PES 30 and PES 50 had a percent transmittance similar to water (100%) and tender coconut water. However, the percent of transmittance did not show a significant difference in the tender coconut water samples. The thermal process used as a comparison also did not show any significant differences for each untreated sample. This shows that the ultrafiltration process can improve the mature coconut water's appearance better than the thermal process. Whereas in tender coconut water, the ultrafiltration process did not change its appearance much.

The difference in transmittance can also be related to the appearance of coconut water in Figure 3. In Figure 3, the ultrafiltered coconut water has a more transparent appearance than the untreated and thermal process.

The difference in total sugar of tender and mature coconut water was also reported by Jackson *et al.* (2004) and Keng *et al.* (2017). Kannangara *et al.* (2018) reported that in the early stage of coconut fruit growth, the sugar present in the water is mostly reducing sugars (glucose and fructose). However, as the ripening happens, non-reducing sugar (sucrose) starts to dominate and causing a decrease in the sweetness of coconut water (Jackson *et al.*, 2004). Kannangara *et al.* (2018) also said that solid endosperm would adsorb solutes in coconut water during maturation. Table 1 also shows an increase in sodium value on all mature coconut water compared

to tender coconut water. A similar trend was reported by Tan *et al.* (2014). According to Villanueva *et al.* (2004), migration of inorganic substances occurs from different plant parts to the region of active growth like fruit during the maturation process.



Figure 3. The appearance of tender and mature coconut water before and after ultrafiltration using polyethersulfone membrane. TCC: untreated tender coconut water, TC30: tender coconut water treated with PES 30, TC50: tender coconut water treated with PES 50, TCT: tender coconut water heated at 90°C for 10 mins, MCC: untreated mature coconut water, MC30: mature coconut water treated with PES 30, MC50: mature coconut water treated with PES 50, MCT: mature coconut water heated at 90°C for 10 mins.

3.3 Antioxidant activity

The impact of ultrafiltration and thermal processes on the antioxidant activity of tender and mature coconut water can be seen in Table 2. The table shows that the ultrafiltration process caused significantly lower ($P<0.05$) in the total phenolic and RSA levels of coconut water compared to the untreated and thermal process. The TPC of untreated tender and mature coconut water in this study is similar to Arzeta-Ríos (2020) that accounted for 4.60 and 6.91 mg GAE/100 mL, respectively.

Decreases in TPC and RSA were also observed in research by Laorko *et al.* (2010) on pineapple juice processing using ultrafiltration membranes with MWCO 100 and 30 kDa. Laorko *et al.* (2010) suspected that some polyphenols bind to other components in pineapple

Table 2. Total phenolic content and radical scavenging activity of tender and mature coconut water, after filtration with PES 30, PES 50, and thermal process

Sample	TPC (mg GAE/100 mL)	RSA (%)
TCC	5.22±0.58 ^a	70.78±3.90 ^a
TC30	2.99±0.32 ^b	15.95±3.04 ^d
TC50	2.48±0.14 ^b	15.64±5.88 ^{cd}
TCT	4.94±1.21 ^a	46.49±3.09 ^b
MCC	6.00±1.33 ^a	62.97±4.09 ^b
MC30	2.82±0.85 ^b	13.50±1.67 ^d
MC50	3.15±1.52 ^b	15.89±1.89 ^d
MCT	5.21±0.09 ^a	34.14±3.54 ^c

TCC: untreated tender coconut water, TC30: tender coconut water treated with PES 30, TC50: tender coconut water treated with PES 50, TCT: tender coconut water heated at 90°C for 10 mins, MCC: untreated mature coconut water, MC30: mature coconut water treated with PES 30, MC50: mature coconut water treated with PES 50, MCT: mature coconut water heated at 90°C for 10 mins. Values are expressed as mean±SD. Values with different superscript in the same column are significantly different ($P < 0.05$) between samples.

juice so that the molecular weight becomes more massive and is retained on the membrane. However, a different experiment in this study shows that the TPC of retentate in mature coconut water treated using PES 30 also decreased, although not as high as the decrease in permeate. TPC in control, retentate, and permeate of mature coconut water in separate experiments were 5.68; 4.95; and 4.02 mg GAE/100 mL respectively. This proves that the decrease in polyphenol was not only due to the molecular weight but presumably also due to the interaction between the membrane and the phenolic compounds in the sample. The same decrease was observed in the research by Mnif *et al.* (2017) using phenol and polyethersulfone membranes. It was reported that there was an interaction effect between the membrane and the solute during the ultrafiltration process, and it is suspected that this happened because of the adsorption on the PES membrane.

An infrared assay using FTIR was conducted further to validate the phenomenon of TPC and RSA decrease. Six PES membranes were analyzed including 30 B (untreated PES 30 membrane), TC 30 A (PES 30 after ultrafiltration of tender coconut water), MC 30 A (PES 30 after ultrafiltration of mature coconut water), 50 B (untreated PES 50), TC 50 A (PES 50 after ultrafiltration of tender coconut water), and MC 50 A (PES 50 after ultrafiltration of mature coconut water). The spectra of each sample are shown in Figure 4.

The spectra in Figure 4 shows a similar peak on the treated and untreated membranes on each MWCO. According to Balasundram *et al.* (2006), a phenolic

compound comprises an aromatic ring bearing one or more hydroxyl groups. The most abundant phenolic compounds found in coconut water were catechin, epicatechin, and salicylic acid, followed by hydroxybenzoic acid, syringic acid, p-coumaric acid, coumaric acid, and cafeic acid in a small amount (Chang and Wu, 2011; Mahayothee *et al.*, 2016). Therefore, the emergence of new peaks in the wavelength range of hydroxyl groups, aromatic rings, and carboxylic acids indicated phenolic compounds retained or bound to the membrane during the filtration process. The new peak at TC 30 A was at a wavelength of 972.12 cm^{-1} (aromatic ring), 1242.16 cm^{-1} (primary or secondary OH), and 2607.76 cm^{-1} (carboxylic acid). While the new peaks at MC 30 A were at wavelengths of 1404.18 cm^{-1} (phenol or tertiary alcohol), 1928.82 cm^{-1} and 1951.96 cm^{-1} (combining aromatic bands), 2630.91 cm^{-1} (carboxylic acid monomers), 2677.2 cm^{-1} (carboxylic acid hydrogen bonds), and 3101.54 cm^{-1} (aromatic rings) (Coates 2006). Changes in peak spectra were also observed in the static polyphenol adsorption experiment by Susanto *et al.* (2009). Susanto *et al.* (2009) reported that the membrane which was exposed to polyphenol compounds for 3 hours and washed showed changes in peak spectra in the range $\sim 3000 - 3700 \text{ cm}^{-1}$ (OH group) which was indicated as a polyphenol compound.

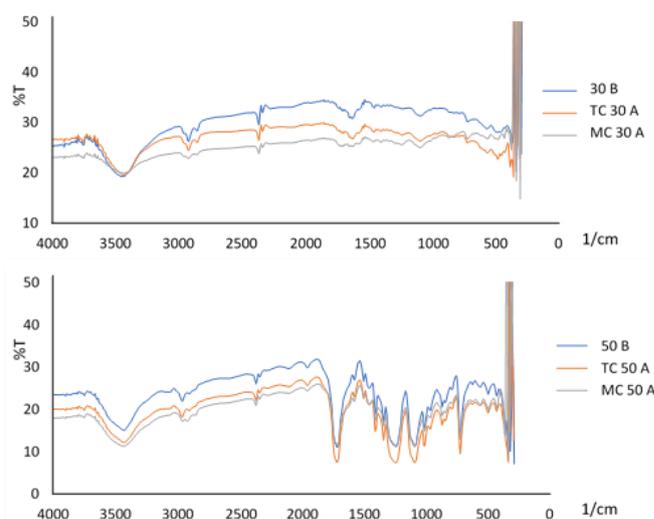


Figure 4. FTIR spectra of PES 30 B, TC 30 A, MC 30 A, 50 B, TC 50 A, and MC 50 A membranes. 30B: PES 30 membrane before ultrafiltration, TC30A: PES 30 membrane after ultrafiltration of tender coconut water, MC30A: PES 30 membrane after ultrafiltration of mature coconut water, 50B: PES 50 membrane before ultrafiltration, TC50A: PES 50 membrane after ultrafiltration of tender coconut water, MC50A: PES 50 membrane after ultrafiltration of mature coconut water.

3.4 Enzyme activity

The effect of ultrafiltration and thermal treatment on the enzymatic activity of tender and mature coconut water can be seen in Table 3. The table shows that

filtration using PES 30 provides a similar inhibition to the thermal process. However, filtration using PES 50 membrane still shows enzyme activity.

The data in Table 3 shows that ultrafiltration using PES 30 can inhibit all POD enzyme activity equivalent to thermal treatment. According to Duarte *et al.* (2002) and Balasubramanian and Boopathy (2013), peroxidase in tender coconut water has a molecular weight of 44.63 kDa, 47 and 49 kDa. At the beginning of the ultrafiltration process using the PES 50, a small portion of the enzyme can pass through the membrane due to vacuum pressure. However, along with the increase in processing time, there was the entrapment of particles in the membrane pores, which can cause shrinkage of the pore size. This causes a decrease in enzyme activity in the permeate. Meanwhile, in the ultrafiltration process using PES 30, the molecular weight of the enzyme was much different from the size of the membrane, which causes no enzyme to pass through the membrane.

Table 3. The activity of peroxidase and polyphenol oxidase enzymes in control of tender and mature coconut water, filtration with 30 kDa PES membrane, 50 kDa PES, and thermal

Sample	POD		PPO	
	Activity (U.ml ⁻¹)	Retention (%)	Activity (U.ml ⁻¹)	Retention (%)
TCC	19.23±10.27 ^b	-	7.54±3.24 ^a	-
TC30	ND	100	1.05±0.14 ^b	86.07
TC50	0.23±0.32 ^a	98.8	3.80±3.75 ^{ab}	49.6
TCT	ND	100	ND	100
MCC	68.75±0.43 ^c	-	ND	100
MC30	ND	100	ND	100
MC50	0.15±0.21 ^a	99.78	ND	100
MCT	ND	100	ND	100

TCC: untreated tender coconut water, TC30: tender coconut water treated with PES 30, TC50: tender coconut water treated with PES 50, TCT: tender coconut water heated at 90°C for 10 mins, MCC: untreated mature coconut water, MC30: mature coconut water treated with PES 30, MC50: mature coconut water treated with PES 50, MCT: mature coconut water heated at 90°C for 10 mins, ND: Not detected. Values are expressed as mean±SD. Values with different superscript in the same column are significantly different (P<0.05) between samples.

The data in Table 3 shows that the ultrafiltration treatment in tender coconut water still shows PPO enzyme activity in the permeate. In contrast, the thermal process did not show any PPO enzyme activity. A decrease in the PPO and POD enzyme activity by 95.10% and 97.89% in tender coconut water was also observed in the study by Lamdande *et al.* (2020) using a PES 30 kDa membrane. Treatment of tender coconut water using a flat sheet membrane with an MWCO of 20 kDa also decreases 95.99% (Nakano *et al.* 2011).

Meanwhile, Debien *et al.* (2013) reported that an ultrafiltration process using 30 kDa Regenerated Cellulose (RC) could completely inhibit PPO and POD enzyme activity. The different types of membranes used can provide different particle containment percentages. Schmidt *et al.* (2016) reported the use of PES membranes showed protein levels in retentate two times lower than the use of RC membranes that shows the PES membrane has a more open structure compared to RC. In addition, different manufacture of membranes can also produce asymmetric pores that are not always the same (Galanakis, 2015; Tang *et al.*, 2015)

The PPO enzyme in coconut water has a molecular weight of 64.8 kDa (Demir *et al.*, 2012). Although PPO has a molecular weight much larger than the MWCO of the membrane, there is still enzyme activity in the permeate. A similar phenomenon was observed by Butterworth *et al.* (1970) in α -amylase filtration. According to Schmidt *et al.* (2016), it was suspected that the escape of enzyme could also be caused by the enzyme not being in a complex form but in the monomer form so that it had a lower molecular weight.

4. Conclusion

Ultrafiltration using polyethersulfone membranes with molecular weight cut-off of 30 kDa and 50 kDa reduced the activity of PPO and POD enzymes, improved clarity, and maintained most chemical characteristics of tender and mature coconut water. Processing using PES 30 kDa had a higher permeate flux and better enzyme removal than that of PES 50 kDa. However, the ultrafiltration process in this study was not able to maintain the total phenolic content and antioxidant activity of coconut water, therefore further experiment is still needed.

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