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RESEARCH ARTICLE

Cold sterilization of coconut water by membrane technology and UV-C

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OBJECTIVES Coconut water contains amino acids, vitamins, antioxidants, and minerals, which benefit human health. However, the rapid degradation of coconut water due to the presence of protein, fat, and microbes has led to short shelf life and coconut water's rancidity. Sterilization of coconut water by heat treatment has been proven effective in microorganism elimination but results in major changes in coconut water's sensory attributes. METHODS In this research, cold sterilization of coconut water was conducted using an ultrafiltration membrane and UV-C to preserve coconut water's sensory properties and nutritional components. The radiation dose of UV-C and the operating pressure of the ultrafiltration membrane were varied to obtain the optimum operating condition. RESULTS The sterilization process by UV-C did not remove fat and protein, which are the rancidity-causing components. In ultrafiltration sterilization, fat and protein can be removed by 74% and 31.37%. Superior microorganism elimination by ultrafiltration was obtained at 99.9999%, compared to UV-C at 90%. The ultrafiltration also retained the coconut water's pH, total soluble solid, and flavor while improving its clarity. CONCLUSIONS At an optimum operating pressure of 0.25 bar, the coconut water's shelf life of 3 days, according to the Standar Nasional Indonesia (SNI), can be achieved.

KEYWORDS coconut water; microorganism; sterilization; ultrafiltration; UV-C

1. INTRODUCTION

Coconut water is a source of vitamin B complex, vitamin C, minerals, and amino acids, with numerous benefits for human health. In particular, the minerals in coconut water can regulate heart rate, muscle and nerve function (Reddy and Lakshmi 2014). Coconut water also contains enzymes such as polyphenol oxidase (PPO) and peroxidase (POD). Enzyme activity in coconut water can cause color changes that can affect the nutrition, taste, and color of coconut water (Prades et al. 2012). PPO enzymes work optimally at pH 6.0 and 25 °C, and POD enzymes at pH 5.5 and 30 °C. In addition, coconut water contains fats and proteins that can deteriorate the coconut water in long-time storage. The fatty acids in coconut water can be oxidized to produce alcohol compounds that can cause a rancid smell in coconut water. Microbes, such as *E. colli, L. monocytogenes, L. rhamnosus, Salmonella typhimurim*, etc, are also found in coconut water. To slow down the deterioration of coconut water, processing is needed to extend the shelf life of coconut water. A common method to extend the shelf life of coconut water is the refrigeration of coconut water. However, while refrigeration prevents the growth of microorganism, the reduction of microorganisms is improbable, and the possibility of degradation is still high (Erkmen and Bozoglu 2016). In addition, when coconut water is frozen, consuming coconut water will be difficult because it must be thawed. Another method that can be done is sterilization, either thermally or non-thermally.

Sterilization is a process that aims to destroy spoilage and pathogenic microbes. Based on the Sterility Assurance Level (SAL), sterilization is characterized by a decrease in microbes by 12 log cycles (Sandle 2013). The most frequently used methods for sterilizing beverages are pasteurization and HTST (Dobson et al. 2017). Both methods use heat to remove microbes. However, these two methods are unsuitable for coconut water because they can alter the taste and flavor of coconut water. Non-thermal sterilization methods can be applied to sterilize coconut water. The use of non-thermal sterilization in the food industry is starting to be widely used. Some non-thermal sterilization methods are high-pressure processing (HPP), plasma bubbling, ultrasound, UV light, etc. A study by Cappelletti et al. (2015) demonstrated the pasteurization technique using the high-pressure carbon dioxide (HPCD) method and its effect on coconut water's nutri-

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tion and sensory quality. The carbon dioxide used for HPCD is in liquid state with a concentration of 99.99% and a flow rate of 6 MPa/minute. Coconut water was processed under 8 and 12 MPa, at temperatures 22, 30, 35, 40, and 45 °C for 5-60 minutes. Meanwhile, the pasteurization process was performed at 72 °C for 5 and 10 minutes and 90 °C for 1, 3, 5, 10, and 20 minutes. The results indicated that the number of microbes decreases when the processing temperature increases. At a pressure of 8 Mpa, mesophilic microorganisms achieved a decrease in the number of 4 logs at 45 °C for 30 minutes and 35 °C for 60 minutes. The mesophilic bacteria required 45 minutes of processing at both pressures to make their numbers undetectable. Meanwhile, inactivation of coliform bacteria took 45 minutes at a pressure of 8 MPa and 30 minutes at a pressure of 12 MPa. Color changes occur in both HPCD processing and pasteurization. Colorimetric results show E of pasteurization 8.1 and HPCD 5.1; therefore, it was concluded that the color change of pasteurization processing was more significant than that of HPCD, which might be caused by emulsion destabilization and protein precipitation.

The difference between thermal and non-thermal processing results can be seen in the research conducted by Ma et al. (2019) comparing the effects of High-Pressure Processing (HPP) and High-Temperature Short Time (HTST) on the number of bacteria and the organoleptic properties of coconut water. HPP was carried out with HPP-650, which reached a process pressure of 500 MPa, while HTST was carried out at a temperature of 72 °C for 15 seconds. The number of bacteria in HPP coconut water was more stable for 15 days of storage than HTST. Color changes analyzed using colorimetry also showed that HPP decreased the L value and increased the b value, while HTST increased the redness of coconut water more significantly than HPP. The color of coconut water from HPP processing is more stable than that of HTST during storage. The number of amino acids and proteins in coconut water in both processing did not change significantly. As for the taste of coconut water, according to 20 panelists, coconut water processed by HPP is more similar to fresh coconut water than coconut water from HTST.

The processing of coconut water using UV-C light to extend shelf life has attracted many researchers. UV-C light has been shown to reduce the number of bacteria in coconut water and extend the shelf life of coconut water by 1.5 - 4 times longer than coconut water that is not exposed to UV-C (Donsingha and Assatarakul 2018). However, The UV-C treatment cannot remove fat and protein in coconut water, which may cause deterioration and rancidity in coconut water (Handojo et al. 2012). Therefore, research on the application of membrane technology for coconut water processing have gain much interest. The microfiltration and ultrafiltration of coconut water successfully decrease the number of microbes, nutrient content, and sensory properties of coconut water (Handojo et al. 2012; Karmakar and De 2017). The nutritional content of coconut water processing with membranes is influenced by different operating pressures (Handojo et al. 2012) and flow rates (Karmakar and De 2017). While the sensory properties of coconut water have decreased but are not significant, it is still favored by panelists (Karmakar and De 2017). Another study by Nakano et al. (2011) compared coconut water without processing, with membranes, and with pasteurization (thermal). The analysis carried out on the three variations are pH, total soluble solid, acidity, enzymes, and microbes. The experimental results show that the pH, TTS, and acidity of the three variations were similar. The results of the microbial analysis showed that coconut water processing by membranes and pasteurization met the safe limits of microbes.

This study aims to extend the shelf life of coconut water with combined non-thermal sterilization methods, which are ultrafiltration and UV-C rays. UV-C treatment was conducted to reduce the microbes, while the microfiltration was conducted as a further sterilization method, as well as removed the fat and protein in the coconut water. The sterilized coconut water is expected to be free from fat, protein, and microbes, and maintain the sensory quality of coconut water. Changes in pH, Total Soluble Solid (TSS), and ion content in coconut water after processing and storage were also analyzed to determine the effect of processing on the nutritional content of coconut water. This research is expected to determine the most suitable UV-C dose to sterilize coconut water and determine the operating pressure of the ultrafiltration membrane that best removes protein, fat, and microbes from coconut water.

2. RESEARCH METHODOLOGY

2.1 Materials

Fresh coconut water was used as the feed in all experiments and purchased from local market in Bandung, Indonesia. The coconut water is immediately stored in the refrigerator as soon as it is removed from the fruit, without further treatment.

2.2 Procedures

2.2.1 UV-C Sterilization of Coconut

Two hundred mL of coconut water was filtered using a filter cloth and placed in a container. Next, the pH and TSS were measured. A container filled with coconut water was put into a pre-assembled UV-C sterilization set-up (Phillips UV-C disinfection lamps), with an irradiance of 28 µW/cm^2 when put in the distance of 1 m. The distance between the UV-C source and coconut water was set to determine the dose of UV-C light (Figure 1(a)). UV-C was operated with time variations of 5, 10, 15, and 20 minutes. Sample of coconut water was taken according to the variation, and the pH and TSS were measured. For microorganism quantification, coconut water was put in sterile vials and stored in a refrigerator (8 °C) for 12 hours before characterization.

2.2.2 Ultrafiltration of Coconut

Ultrafiltration was carried out using a commercially available PVDF hollow fiber membrane with a pore size of 0.01 m and surface area of 0.7 m^2 (Aquatech Water Filter). The feed pressure is set at predetermined variation, 0.25 bar, 0.5 bars, and 0.8 bar, by adjusting the valve opening on the retentate flow. The retentate flow is then recycled back into the feed tank, while the permeate flow is collected in the permeate tank (Figure 1(b)). The permeate was stored to analyze protein, fat, total soluble solids, pH, and ion content. The permeate from ultrafiltration at a pressure of 0.8 bar was partially separated

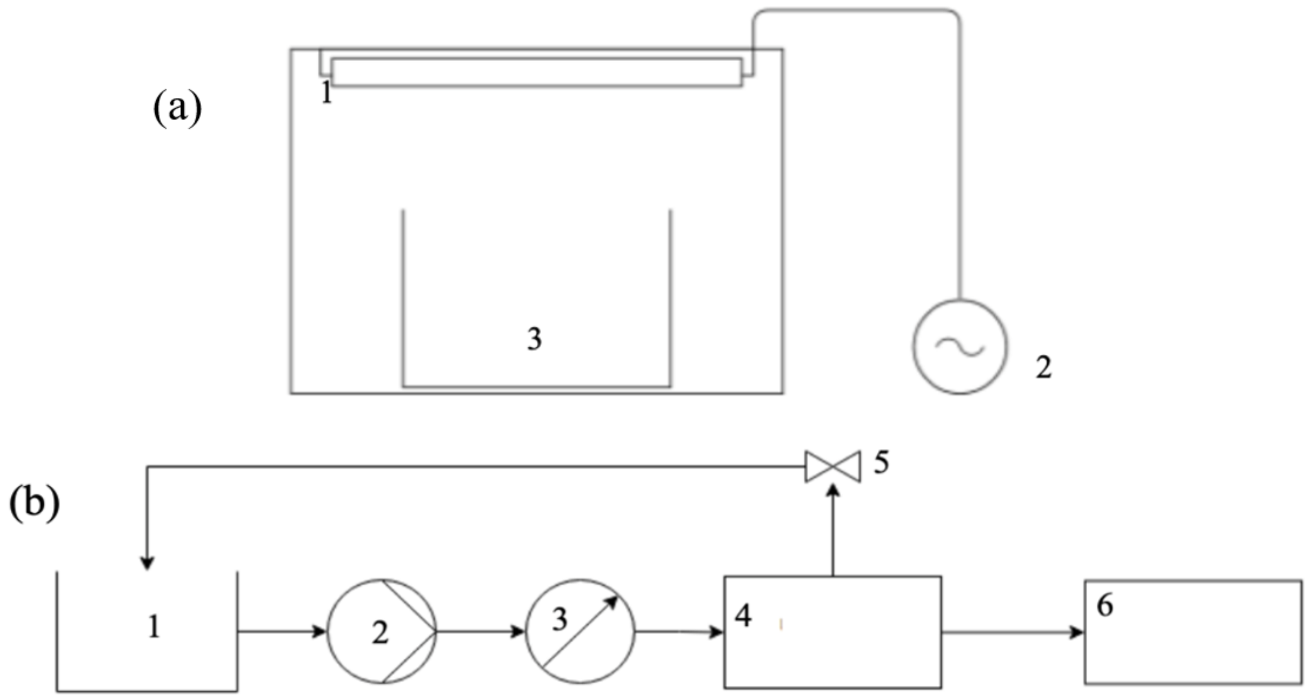

FIGURE 1. Experimental set-up of (a) UV-C (1-UV-C lamp, 2-electrical source, 3-coconut water tank) and (b) UF (1-coconut water feed tank, 2-pump, 3-pressure gauge, 4-UF membrane, 5-retentate valve, 6-permeate tank).

to be sterilized using UV-C light.

2.2.3 characterization

A. Protein Content (Kjeldahl, IK-SBWH-02-N-II)

Protein testing was carried out by the Bandung Sibaweh Laboratory. A total of 0.51 grams of the sample was put into a Kjeldahl flask. A total of 2 grams of selenium mixture and 25 mL of H_2SO_4 were mixed into a Kjeldahl flask. The sample mixture was then heated to boiling for 2 hours, and the color changed to clear greenish. Afterward, the mixture was cooled and put into a 100 mL volumetric flask. A total of 5 mL of the cold mixture was pipetted, and 5 mL of 30% NaOH and a few drops of PP indicator solution were added. Titration was then conducted using 0.01 N HCl solution.

B. Fat Content (Soxhlet, SNI 01-2891-1992 Item 8.1)

The fat characterization was carried out by the Bandung Sibaweh Laboratory. A total of 2 grams of the sample was put into a paper-lined container covered by cotton. The samples were then dried in an oven at a temperature of 80 °C for one hour. The dry sample is then put into a Soxhlet apparatus connected to a flask containing boiling stones that have been dried and weighed. Extraction using hexane solvent was carried out for 6 hours then the hexane was separated from the fat extract. The fat extract was then dried in a drying oven at 105 °C. The dry fat extract was then cooled and weighed. The fat percentage is obtained by subtracting the mass of the sample from the mass of fat before extraction, then dividing the mass after extraction, and multiplying by one hundred percent.

Ion

The ion content of coconut water was characterized by electrical conductivity parameters using an EC meter (CT-3030 Digital Portable Tester). The higher the electrical conductivity value, the higher the ion content in coconut water. Tests were carried out for samples at 1, 5, and 9 days of storage.

pH

The pH of coconut water was measured using an electric pH meter (AS218 Smart Sensor pH Meter) calibrated using a buffer solution of pH 4 and 7. Tests were carried out for samples at 1, 5, and 9 days of storage.

Total Soluble Solid

The total soluble solid of coconut water was tested using a portable TDS meter (EZ-9909). Tests were carried out for 1, 5, and 9 days of storage.

Microorganism Quantification

The microorganism quantification was carried out by Microbiology Laboratory, Institut Teknologi Bandung. A total of 5 grams of tryptone; 2.5 grams of yeast extract; 1 gram of dextrose; 15 grams of agar; and 1 liter of demineralized water were heated to dissolve all the ingredients. The mixture was poured into a petri dish. Then the petri dish was autoclaved for 15 minutes at 121 °C. A 1 mL sample was taken with a micropipette and then put in a 10 mL volumetric flask. The solution was taken with a dropper, then spread on agar media, and allowed to stand for two days at a temperature of 25°C. Afterward, microbes in a petri dish containing blanks and coconut water samples were counted.

3. RESULTS AND DISCUSSION

3.1 Effectiveness of UV-C for coconut water sterilization

The experiment was conducted with UV-C doses of 168 and 336 mJ/cm^2. Coconut water without treatment and treated with UV-C was tested for microorganism content (Table 1). A decrease in the number of microbes in coconut water that has been irradiated was observed. Based on the experiment, UV-C with varying doses could only reduce 0.65 and 1 cycle log of microbes, respectively. The microbial reduction was much less significant than that in the experiment by Bhullar et al. (2018) and Ochoa-Velasco et al. (2018), which indicated

TABLE 1. Microorganism quantification, pH, and TSS of fresh and UV-C treated coconut water.

Treatment	Microorganism quantification (x10^7 colony/mL)	pH	TSS (°Brix)
No treatment (fresh)	32	5	4.2
UV-C 168 mJ/cm^2	6	5	4.2
UV-C 336 mJ/cm^2	3.7	5	4.2

4-6 log cycles and 3-7 log cycles reduction of microorganism content. The ineffectiveness of the UV-C irradiation may be due to differences in equipment and working methods. In the literature, coconut water is irradiated by flowing coconut water so that coconut water forms a thin layer (flowing in a 0.5 cm diameter hose), whereas, in this study, coconut water is neither flowed nor agitated and has a thickness of >2 cm. Coconut water that does not flow/agitated resulted in the varied UV-C dose at every part of the coconut water. This affects the ability of UV-C to sterilize coconut water. In addition to the microorganism quantification, pH and TSS were also analyzed. The pH and TSS of coconut water did not change significantly after UV-C irradiation. This is in accordance with the statement of Erandya Jayawardena et al. (2019) and Gunathunga et al. (2018) that the pH and TSS of coconut water that has been through UV-C radiation did not change significantly. In addition, there was no visible color change in coconut water before and after UV-C irradiation. Coconut water remains colorless and cloudy before and after UV-C irradiation. In contrast, the change in the taste of coconut water before and after UV-C treatment was observed. Coconut water irradiated with UV-C rays tasted bitter and had a yeasty flavor aftertaste.

3.2 Ultrafiltration of Coconut Water

During the processing and storage of coconut water, changes in ion content, pH, TSS, and microorganism content were observed. Another thing that can be observed when processing coconut water is the change in the permeate flux during ultrafiltration (Figure 2). According to Figure 2, coconut water permeate flux was stable, and membrane fouling had not occurred due to the short operation time. The flux of the operations was not affected by the feed pressure, as indicated by comparable initial flux for operations at varied feed pressure.

The protein and fat content in coconut water can shorten the shelf life of coconut water, so ultrafiltration is expected to reduce the protein and fat content. The protein and fat content in UF-treated coconut water are shown in Figure 3. The measurements were taken at the end of the experiments, and the samples were the total permeate of the experiments. As described in Figure 3(a), the protein content of coconut water after processing through ultrafiltration membranes was reduced. At a pressure of 0.25 bar, 0.5 bar, and 0.8 bar, the protein rejection was 31.37%, 27.45%, and 15.59%, respectively. These results indicate that the size of the protein molecule is larger than the membrane's pore size and causes the protein to be retained and not enter the permeate stream. According to the results in Figure 3(b), the fat content of coconut water after processing through ultrafiltration membranes was reduced. At a pressure of 0.25 bar, 0.5 bar, and 0.8 bar, the fat rejection was 60%,70%, and 74%, respectively. These results indicate that the size of the fat molecule is larger than the membrane's pore size and causes the fat molecule to be retained and not enter the permeate.

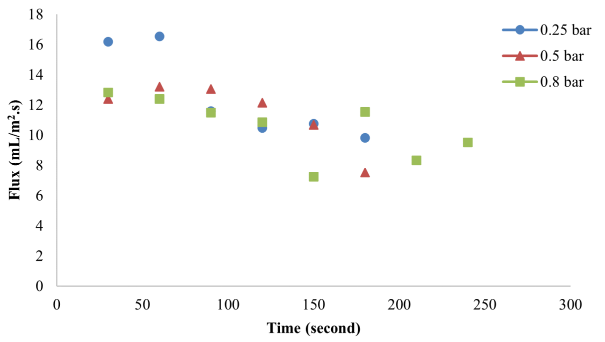

FIGURE 2. Flux profile of coconut water ultrafiltration at varied feed pressure.

3.3 Physical properties and microbial contamination of sterilized coconut water

3.3.1 Ion content

Coconut water has a high ionic content, making it a good source of electrolytes. In processing coconut water, the ion content shouldn't change to get the maximum benefit of coconut water. In this study, observations were made of the ion content of coconut water that had been treated by ultrafiltration and ultrafiltration followed by UV-C irradiation. The results of the observations are shown in Figure 4. Electrical conductivity parameters convey the results of the ion content, as ions in water can conduct electricity. The drawback of measuring the electrical conductivity parameter is that it is impossible to know the type of ion being measured. Based on the results in Figure 4, the ion content of coconut water did not decrease after ultrafiltration at a pressure of 0.5 and 0.8 bar. This shows that the ions easily pass through the membrane pores at a pressure of 0.5 and 0.8 bar. Meanwhile, at 0.25 bar feed pressure, the ion content of coconut water decreased slightly or was rejected by 12.15%.

According to Reddy et al. (2007), processing coconut water using 0.2 µm microfiltration can reduce the ion content of coconut water by 5% and even more when using ultrafiltration membranes. Some rejected ions in microfiltration/ultrafiltration are monovalent (Na and K), and many divalent ions (Mg, Fe, and Cu). In the results of 0.25 bar operation, the rejection is much greater than the coconut water produced by microfiltration in Reddy et al. (2007). This may be due to the contamination of the rinse water in the ultrafiltration apparatus, causing the permeate to become more dilute. During storage, the ion content of the untreated coconut water and coconut water treated by ultrafiltration 0.8 bar + UV-C showed no significant changes. However, there was a decrease in the number of ions in the ultrafiltration at 0.25 bar, 0.5 bar, and 0.8 bar. The decrease in electrical conductivity may occur due to the coconut water fermentation. Changes in the aroma and taste of coconut water character-

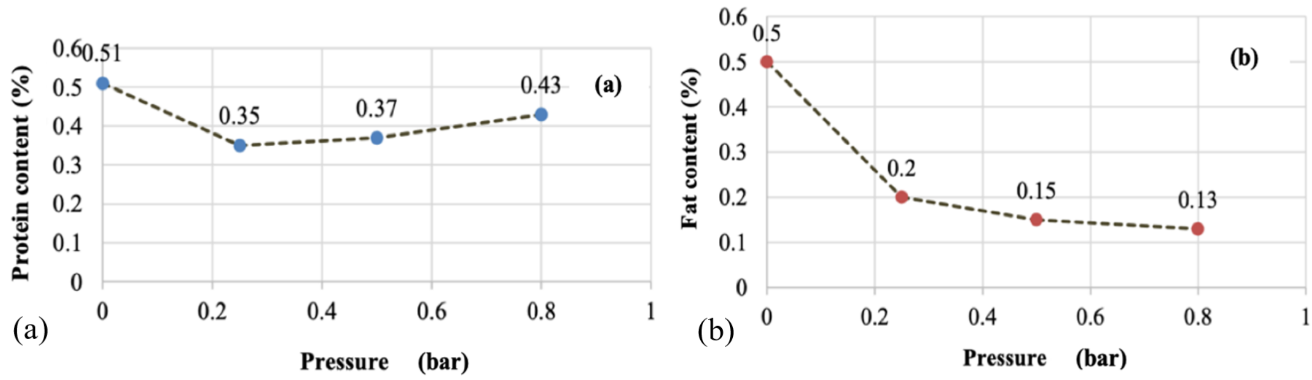

FIGURE 3. (a) Protein and (b) fat content of fresh and ultrafiltration-treated coconut water.

ize the coconut water fermentation process. Fermentation produces alcohol, inhibiting electrical conductivity.

3.3.2 pH profile

The pH of coconut water is one of the parameters for coconut water consumption. Indonesia does not have a coconut water pH standard. However, some countries, such as the Philippines and Jamaica, have national standards for the pH of coconut water. According to the Philippine national coconut water standard (PNS/BAFPS 28: 2006), the standard pH range of coconut water is 4.6-5.1. Meanwhile, according to the Jamaican national standard (DJS CRS 3: 2010), the pH of coconut water is 4.6-5.5. The pH profile of coconut water during storage in this study is shown in Figure 5.

According to Figure 5, the pH of untreated and treated coconut water showed a gradual reduction. During the storage period, the most extreme decrease in pH occurred in untreated and coconut water treated by ultrafiltration of 0.8 bar + UV-C, while a less significant decrease in pH occurred in coconut water resulting from ultrafiltration of 0.25 bar. This may be due to the metabolism of microbes in coconut water to produce acid. The microbes present in untreated coconut water have the highest number, so the decrease in pH due to acid production by microbes is extreme. On the other hand, coconut water resulting from ultrafiltration of 0.25 bar has the least microbial content, so the decrease in pH is not extreme. Based on the pH standards from the Philippines, Jamaica, and India, coconut water treated by ultrafiltration 0.8 bar + UV-C was no longer suitable for consumption on the 5th day. Meanwhile, the untreated coconut water, 0.5 bar ultrafiltration, and 0.8 bar ultrafiltration were unsuitable for consumption on the 6th day.

3.3.3 Total Soluble Solid (TSS) profile

The content of dissolved solids in fruit juice drinks is one of the important parameters in Indonesia. However, in SNI 3719:2014, coconut water does not have a standard, so comparisons are made with standards in other countries such as the Philippines and Jamaica. According to the Philippine national coconut water standard (PNS/BAFPS 28: 2006), the standard range of TSS of coconut water is 4-7.5. Meanwhile, according to the Jamaican national standard (DJS CRS 3: 2010), the TSS of coconut water is 3.8-6.9. The results of the analysis of the TSS of coconut water during storage in this study are shown in Figure 6. The TSS of untreated coconut water decreased during storage, while the TSS of treated coconut water remained constant. This was also observed in the experiment conducted by Reddy et al. (2007), which showed similar results. The TSS of coconut water for all variations during the storage period met the Jamaica national coconut water standard (DJS CRS 3: 2010); however, the coconut water treated by ultrafiltration at 0.25 bar did not meet the Philippine national standard. (PNS/BAFPS 28: 2006).

3.3.4 Microorganism contamination

Microorganism contamination in coconut water is one of the important things that must be considered to determine the suitability of coconut water for consumption. According to SNI 3719:2014, for fruit juice drinks, the maximum microbial content in coconut water is 1x10^4. As indicated in Figure 7, the treatment of coconut water with ultrafiltration can re-

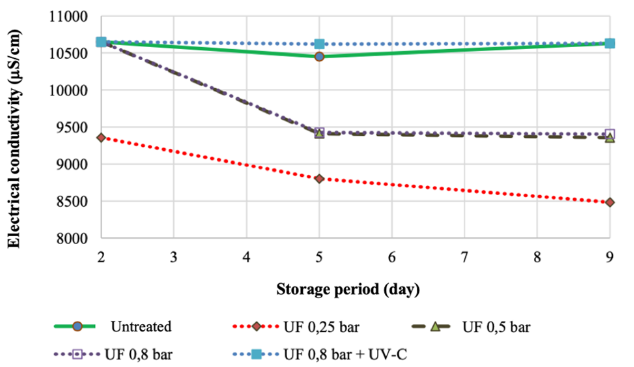

FIGURE 4. Electrical conductivity profile of coconut water during storage.

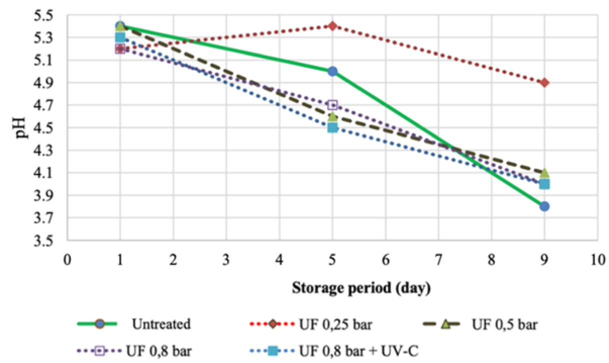

FIGURE 5. pH profile of coconut water during storage.

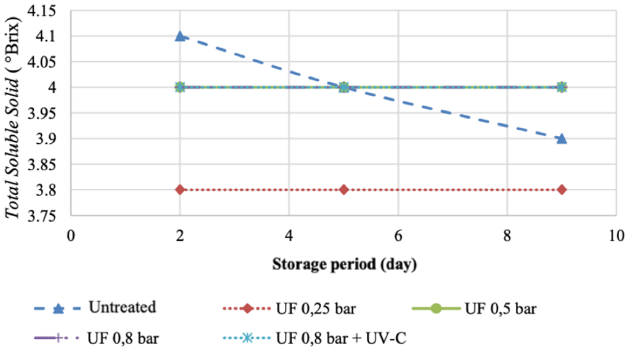

FIGURE 6. TSS profile of coconut water during storage.

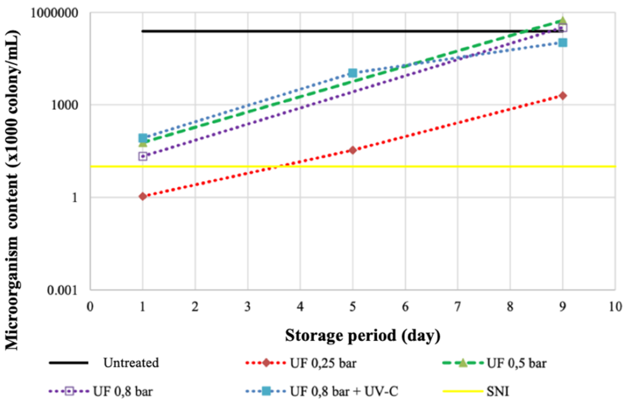

FIGURE 7. Microorganism growth of coconut water during storage.

duce microorganism contamination. Rejection of microorganisms by ultrafiltration with a pressure of 0.25 bar, 0.5 bars, and 0.8 bar was 99.9999%, 99.9%, and 99.99% or equivalent to 5.3, 3,6, and 4 log cycles, respectively. For coconut water with additional UV-C irradiation, the microorganism rejection was 99.9%. This indicated that irradiating coconut water with UV-C light at the respective dose was ineffective for sterilization. As previously discussed, the ineffectiveness of UV-C in this study might be caused by the stagnant and thick layer of coconut water that needs to be irradiated. Although the microbial rejection of coconut water after processing was around 99.9%, the processed coconut water did not meet the standard for consumption. Based on Figure 6, it was indicated that the number of microorganisms increased during the storage period and did not meet the SNI standard since 1st day of storage for those treated by ultrafiltration at 0.5 bar, 0.8 bar, and 0.8 bar + UV-C. On the contrary, the coconut water treated by ultrafiltration at 0.25 bar can still be consumed after three days of storage.

4. CONCLUSIONS

This study investigated the ability of ultrafiltration and UV-C irradiation to extend the shelf life of fresh coconut water. It was concluded that UV-C sterilization was effective when conducted under proper operating conditions. The sterilization using UV-C light did not change the fat content, protein, pH, or TSS and only reduced a maximum of 1 log cycle of microorganisms. However, UV-C might change the taste and flavor of coconut water. Sterilization using ultrafiltration prolonged the shelf life of coconut water until three days, according to the Indonesian standard of microorganism content in beverages. Ultrafiltration operation at 0.25 bar feed pressure was optimum for longer shelf life. In addition, ultrafiltration reduced the amount of fat, protein, and microorganisms in coconut water to 74%, 31.37%, and 99.9999%, respectively. Ultrafiltration did not significantly reduce coconut water's pH, TSS, flavor, and taste.

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