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Concentration of lycopene from watermelon juice using cross-flow filtration assisted with diafiltration

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Abstract

Watermelon is a well-known and high-consumed fruit in Vietnam, considered a rich source of bioactive compounds, especially lycopene. The cross-flow filtration coupled with diafiltration was processed to obtain the concentrated lycopene. The microfiltration process was conducted at a temperature of 50-60^{o}C by using a ceramic α-Al_2O_3 membrane with a pore size of 0.08 µm. Permeate was removed while the collected retentate was fractionated into two parts for further experiments. The first part of the retentate was directly centrifuged. The effect of centrifugation time (2, 5, and 8 mins) and speed (1000, 3000, and 5000 rpm) on lycopene content were evaluated. The other part of the retentate was purified by the diafiltration process. The ratio of retentate: water was varied from 1:0.5 to 1:2 (w/w) to determine its influence on the lycopene content. The results showed that the suitable transmembrane pressure was selected at 5 bars. The membrane fouling phenomena was also observed by a rapid decrease in permeate flux in 40 min, followed by a gradual reduction. The suitable condition of centrifugation was processed at 3000 rpm in 5 mins. The centrifuged retentate in 60 mins of microfiltration contained a lycopene content of 146.63±2.76 µg/mL. The retentate: water ratio of 1:0.5 was noted as an effective ratio to purify the lycopene content, achieving 273.42±4.56 µg/mL. The physicochemical properties of the raw watermelon juice and retentate indicated a positive result from the retentate with enhanced total solid content, lycopene concentration as well as purity.

1. Introduction

Bioactive compounds have been gaining worldwide attention due to their beneficial effects on human health. Many studies have been investigated to point out the beneficial values of natural bioactive compounds from fruits and vegetables including flavonoids, polyphenols and essential oils. (Nguyen *et al*., 2020; Mai *et al.,* 2020; Tran, Tran, Nguyen et *al*., 2020; Tran, Dao, Ngo et *al*., 2020). Carotenoids ($C_{40}H_{56}$), among these bioactive compounds, can be found in fruits, vegetables, fungi, bacteria, and animals. These substances create a variety of colours for fishes, birds and insects (Maoka, 2020). Carotenoids are powerful antioxidants in protecting the human body against damaging agents by free radicals, stimulating the immune system by activating natural killer cells as well as killing low–level cancer cells. Besides, they also contribute to reducing cardiovascular diseases, cholesterol in the blood and harmful effects of sunlight on the skin (Chew and Park, 2004; Krinsky, 2004; Gammone, 2015).

Lycopene is a lipophilic red-coloured carotenoid pigment, composed of 8 isoprene units with only carbon and hydrogen (Cámara *et al*., 2013). Lycopene may have a direct effect on reducing the risk of prostate cancer and is considerably more effective against certain types of cancer than any other known nutrients. In addition, lycopene reduces the risk of myocardial infarction, and blood pressure as well as prevents LDL cholesterol oxidation (Przybylska, 2020). Lycopene, naturally located in chromoplasts of plant cells, is primarily found in tomato fruits. Besides, it also can be found in other edible fruits such as watermelon, guava, pink grapefruit and gac fruit. (Cámara *et al.,* 2013). Watermelon is originally cultivated in tropical regions of Africa. Nowadays, it is widely grown in many countries around the world (Naz *et al.,* 2014). In Vietnam, watermelon is considered a major consumed portion, but in the form of fresh fruit rather than processed form. In many crops, when the supply of this fruit is higher than the demand, this causes economic loss, leading to the disposal of

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watermelon fruits. Lycopene concentration from watermelon as a value-added product can be one of the solutions to tackle the problem of inventory obsolescence while enhancing the biological activities of lycopene through the concentration process.

Cross-flow filtration, an alternative approach, has been increasingly applied in fruit processing for the clarification of juices, fractionation or concentration of bioactive compounds (Paes *et al*., 2015). In the filtration process, hydrophobic compounds such as lycopene are normally retained by the membrane due to their attachment to insoluble solids (Chaparro *et al.,* 2016). This technology is considered an advantage in preserving the original nutrients and flavour components by using a low temperature during processing compared to conventional processes which need to be operated at a higher temperature, affecting noticeably the quality of fruit products (Strathmann, 1986). Diafiltration is a variation of cross-flow filtration with the aim to obtain a higher purity of lycopene concentration by washing small molecules as salts away from the retained macromolecules (Chaparro *et al.,* 2016). Two modes can be operated by diafiltration including constant volume diafiltration (CVD) and variable volume diafiltration (VVD). In CVD, the original volume of the system is unchangeable which means that the permeate flow rate was equal to the added water flow rate. In contrast, added water flow rate could be adjusted to either a lower or a higher permeate flow rate depending on the desired concentration of the retentate (Miller and Chappell, 2015).

The efficiency of membrane separation technologies is governed by many parameters: amongst them, the most active are the feed characterizations, the materials constituting the membrane and the hydrodynamics of the whole equipment including transmembrane pressure, filtration time, and feeding flow. Previous studies observed that the permeate flux change over membrane pressure (Youravong *et al*., 2003; Chiu *et al*., 2006; Mai *et al*., 2014). With a further increase in TMP, permeate flux becomes independent of pressure. TMP can also cause a deposit of particles on the membrane surface, which results in fouling and consequently leads to a decreased permeate flux (Kartika, 2005; Cassano *et al*., 2007; Ushikubo *et al*., 2007). One of the major difficulties of the membrane process is fouling which needs to be controlled in order to increase the yield of filtration. Membrane fouling is a limiting phenomenon in filtration technology caused by an accumulation of particles on the membrane surface and results in a decrease in filtration flux (Baker, 2012). Membrane fouling could be due to numerous phenomena including concentration polarization, gel formation, particle adsorption, and pore blockage (Schaller *et al*., 2006).

This paper aimed to evaluate the performance of cross-flow filtration assisted with diafiltration in the lycopene concentration from watermelon fruits. The resulting lycopene concentrate was also investigated for its physicochemical properties.

2. Materials and methods

2.1 Sample preparation

Selected watermelon (*Citrullus lanatus*) of Hac My Nhan variety was supplied from Long An province, Vietnam. The raw fruit, after washing with tap water, was cut and peeled manually to remove the epicarp and seeds. The fruit pulp was then pureed by using a blender and was pre-filtered by a 0.5 mm mesh to remove the residual seeds, resulting in watermelon juice.

2.2 Cross-flow filtration process of watermelon juice

The schematic diagram of the whole process is presented in Figure 1. The filtration process was developed by using the methods of Chaparro with some modifications (Chaparro, 2016). The experiments were designed by using the results of different trial tests. Briefly, the microfiltration process was operated at the temperature of 50-60^{o}C by using a ceramic α- Al_2O_3 membrane (supplied by Atech innovations Gmbh, Deutschland) with a pore size of 0.08 µm, 25 cm in length, 6 mm and 10 mm in inner and outer diameter. The transmembrane pressure (TMP) in the system was varied from 2 to 5 bars to investigate its effect on the permeate flux and the lycopene content of the retentate. The time variation from 10 to 120 mins of microfiltration was conducted to evaluate the permeate flux.

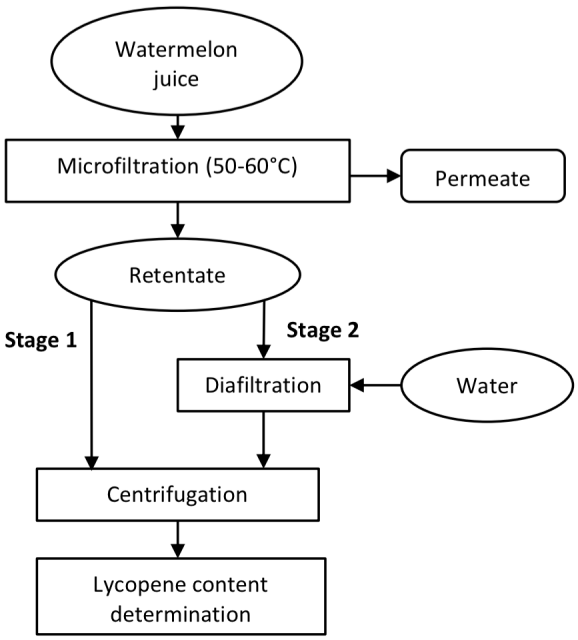

Figure 1. Schematic diagram of microfiltration coupled with diafiltration

After finishing the microfiltration process, permeate was removed while the collected retentate was fractionated into two parts for further experiments. Firstly, the retentate was directly gone through the centrifugation process. The effect of time (2, 5, and 8 mins) and revolutions (1000, 3000, and 5000 rpm) of the centrifuge on lycopene content were evaluated. The other part of the retentate was continued to purify by diafiltration. The retentate: water ratio was varied from 1:0.5 to 1:2 (w/w) to determine its influence on the lycopene content.

2.3 Determination of lycopene content

Lycopene standard was purchased from Sigma-Aldrich, Bangalore, India. The lycopene content in the retentate was determined by using UV-VIS spectrophotometry, complying with the Lambert-Beer law (Priam *et al.,* 2017; Bhattacharjee *et al.,* 2018). The lycopene content is expressed in the below equation:

$$\text{Lycopene content } (\mu g/mL) = \frac{M \times A_{470} \times 10^3}{\varepsilon \times l}$$

Where M is the molecular weight of lycopene (536.9 g/mol), A is the absorbance of lycopene at 470 nm, ε is the molar extinction coefficient of lycopene (1.585×10^5 $M^{-1}cm^{-1}$) at 470 nm and l is the path length of emitted light (cm)

2.4 Determination of permeate flux

The permeate flux (J) was experimentally calculated following the below equation: (Paes *et al.,* 2015)

$$J = \frac{m_p}{A_p.t} \ (l/h/m^2)$$

Where m_p is the mass of permeate in time t (h) and A_p is the area of the membrane (m^2)

2.5 Characterization of physicochemical properties retentate

The watermelon juice and collected retentate were examined to determine their physicochemical properties. The total soluble solids (TSS) were read by using a digital refractometer (Mettler Toledo). Determination of moisture was based on the drying method in the oven at 75°C until getting a constant weight of the sample (AOAC, 2000). The weight loss is the moisture content of the sample. Ash determination was carried out at 550°C in a furnace (AOAC, 2000). The weighed sample was placed in a dried/pre-weighed porcelain crucible. The ash content was considered as the weight difference after weighing the cooled crucible. Colour measurement was determined using a Minolta Chroma Meter and expressed as CIE values of L*, a*, and b* (Rawson *et al.*, 2011).

2.6 Statistical analysis

Each experiment was conducted in three replicates. Statistical analysis was evaluated using SPSS 22.0 software (Chicago, USA) and one-way analysis of variance (ANOVA). Tukey's HSD (Honestly Significant Difference) tests ($p<0.05$) were performed to determine significant differences from each other.

3. Results and discussion

3.1 Effect of the transmembrane pressure on the permeate flux and lycopene content

The influence of transmembrane pressure on the permeate flux and lycopene content during microfiltration is described in Figure 2. Generally, the pressure caused a significant impact on the permeate flux and lycopene content. The permeate flux was found to increase along with the applied pressure, from 57.72±4.31 ($l/h/m^2$) at 2 bars to 92.10±4.56 ($l/h/m^2$) at 5 bars. The result was consistent with reported studies (Wu *et al*., 2007; Chaparro *et al*., 2016; Li *et al*., 2018). The pressure generally is considered a driving force that contributes to the permeate flux (Li *et al*., 2018). Wu *et al*. (2007) indicated that the increased permeate flux (from ~4.5 $l/h/m^2$ to ~11 $l/h/m^2$) was the result of increasing transmembrane pressure (from 0.4 to 0.8 MPa). The lycopene content of the retentate was also found to increase from 26.31±1.32 µg/mL to 36.04±1.19 µg/mL ($p\leq0.05$). This could be explained according to the permeate flux. The increase in the pressure led to the increase in the permeate flux which was equivalent to the more amount of concentrated retentate, resulting in a higher content of lycopene in the retentate. In this study, therefore, the suitable condition of the applied pressure was 5 bars, which was used for further experiments.

The results of ANOVA analysis show that the transmembrane pressure (TMP) affects the flux permeate statistically at 95% confidence ($p<0.05$). LSD analysis comparing the influence of TMP shows that the flux permeate at pressure 5 bar has the largest LS mean and a significant difference compared to flux permeate at other TMP (2; 3; and 4 bar). There was no significant difference in flux permeate at TPM of 2 and 3 bar as well as of 3 and 4 bar. However, there is a significant difference in flux permeate at TMP of 2 and 4 bar. The results presented in Figure 2 and ANOVA analysis showed that TMP did not affect the lycopene content in retentate statistically at 95% confidence ($p>0.05$).

3.2 Effect of filtration time on permeate flux

Figure 3 illustrates the permeate flux changes at different times of the microfiltration process. The permeate flux changes could be clearly observed in two

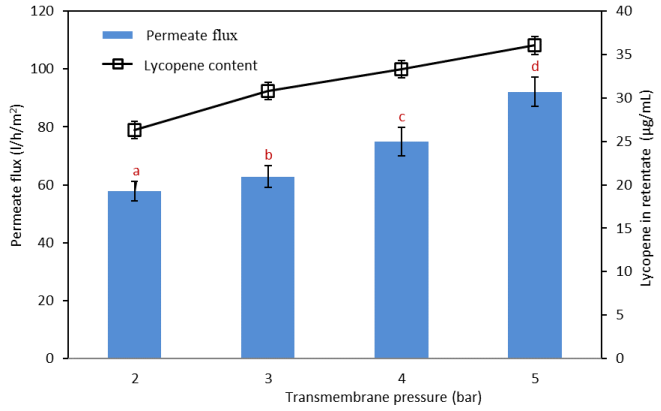

Figure 2. The effect of transmembrane pressure on the permeate flux and lycopene content in microfiltration. Bars with different notations are significantly different ($p<0.05$).

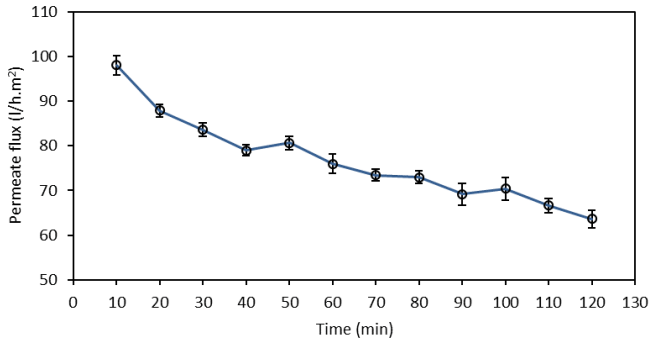

Figure 3. The effect of microfiltration time on the permeate flux. The process was applied a pressure of 5 bars.

stages. The permeate flux experienced a dramatic decline in the first 40 mins, followed by a gradual decrease. The permeate flux at the first 10 mins was recorded at 98.04±2.12 l/h.m^2, decreasing rapidly to 78.94±1.23 l/h.m^2 after 40 mins. A rapid declination of the permeate flux was ascribed to the membrane fouling which macromolecules in the watermelon juice including cellulose, protein, hemicellulose, and colloids deposited and induced a polarized layer. The longer time of filtration facilitated them to deposit and create a second layer in the membrane. This layer then played a role in dictating a slight decrease in the later stage (Rai *et al.,* 2007). A similar flux trend was found in the study of Saleem *et al.* (2017) which was described as a rapid decline in a few minutes and then a gradual decrease in permeate flux (Saleem *et al.,* 2017). Bhattacharjee *et al.* (2018) revealed the same trend of decreasing permeate flux regardless of differences in applied pressure. The permeate flux was stable with a very low decreasing rate during the processing time. This is because of a slow increase in feed concentration and viscosity. The major factors influencing permeate flux were the reversible concentration polarization related to feeding concentration and viscosity, not the irreversible fouling such as internal plugging, adsorption, gel formation and more.

3.3 The effect of the centrifugation process on the lycopene content of the retentate

The effect of centrifugation speed and time of centrifugation is presented in Figure 4.

ANOVA results show that the time and speed of centrifugation affected the lycopene content statistically at the 95% confidence level ($p<0.05$). LSD test to compare the effect of centrifugation speed found that the speed of 3000 rpm has the largest LS mean and is significantly different from the speed of 1000 rpm and 3000 rpm. However, there was no significant difference between the centrifugation speed of 1000 rpm and 5000 rpm. LSD test to compare the effect of centrifugation time found that the time of 5 mins has the largest LS mean and is significantly different from the time of 5 mins and 8 mins. However, there was no significant difference between 2 mins and 8 mins.

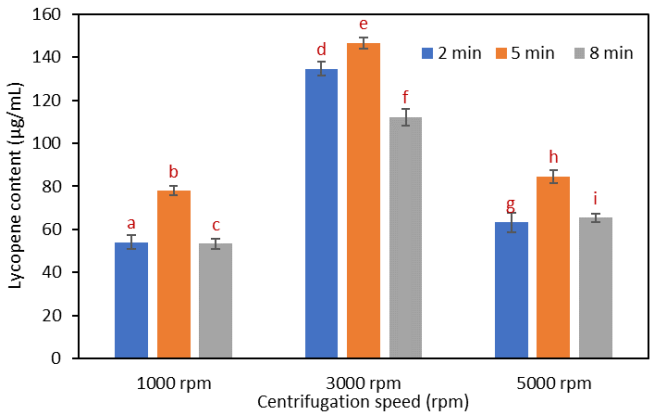

Figure 4. The effect of centrifugation speed and time of centrifugation on lycopene content. The retentate was collected after 60 min of microfiltration at the pressure of 5 bars. Bars with different notations are significantly different ($p<0.05$).

The centrifugation speed reflected how lycopene readily sediments. Normally, small molecular particles are sediment slowly under low centrifugal force, elucidating the low content of lycopene at the speed of 1000 rpm. The highest lycopene content was obtained at the centrifugation speed of 3000 rpm. The speed at 3000 was a considerably adequate centrifugal force to sediment lycopene molecules, leading to better efficiency in obtaining lycopene (Orellana-Palma *et al.,* 2020). However, the very high speed of centrifugation (5000 rpm) was found to cause a negative effect on lycopene content. This could be attributed to the heat generation at a very high speed, which led to the decomposition of lycopene compounds, lowering the lycopene content (Khaksar *et al.,* 2019).

In this studied condition, the centrifugation time of 5 mins was found to be an effective time, achieving the highest lycopene content regardless of different centrifugation speeds. A short period of 2 mins was not

sufficient to sediment these lycopene compounds. Operating the centrifugation process for up to 5 mins resulted in the highest achieved lycopene content. However, the extended time of centrifugation (8 mins) was possibly associated with heat generation, leading to a lower efficacy in the sedimentation of lycopene compounds. As a result, the highest lycopene content was achieved at the centrifugation speed of 3000 rpm in 5 mins, 146.63±2.76 µg/mL, P≤0.05. This study suggested that the extraction of bioactive compounds such as lycopene should be selected at suitable conditions of centrifugal force and adequate time.

3.4 Effect of added water amount on the lycopene content in diafiltration

The effect of added water content on the retentate during the diafiltration process is shown in Figure 5. Diafiltration is generally used to purify the concentration of bioactive compounds, which is usually added to the concentration process (Servent *et al.,* 2020). The higher ratio of water to retentate caused the lower content of lycopene due to a dilution effect which caused a difference in concentration of retentate after microfiltration and retentate after adding water. Therefore, adding water contributed to reducing concentration due to a decrease in the concentration of dry matter in the filtrate. In this study, the better-obtained lycopene content was 273.42±4.56 µg/mL at the retentate: water ratio of 1:0.5. Increasing this ratio up to 1:1.5 significantly caused a reduction in lycopene content, 137.06±5.76 µg/mL.

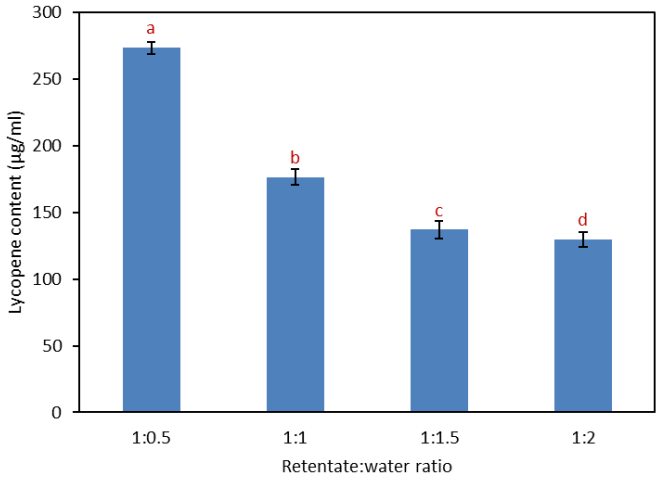

Figure 5. The effect of retentate: water ratio on the lycopene content during diafiltration. Bars with different notations are significantly different (p<0.05). The microfiltration was conducted at 5 bars for 60 mins prior to the diafiltration process. The lycopene content was determined after centrifugation for 5 mins at 3000 rpm.

The ANOVA shows that the ratio of water added to retentate affects the lycopene content statistically at the 95% confidence level (p<0.05). The LSD test to compare the effect of the ratio of water added to retentate found that the ratio of 1:0.5 (v/v) has the largest LS mean and a significant difference with other ratios of 1:1; 1:1.5 and 1:2, v/v. However, there is no significant difference in the ratio of water added to the retentate of 1:1; 1:1.5 and 1:2, v/v. This result is similar to the survey of Chaparro (2016) which showed the optimal retentate: water ratio of 0.77. The higher ratio of water added, the lower the lycopene concentration in the retentate, which reduces the viscosity of the retentate leading to an increase in flux permeating through the membrane. In addition, the addition of water may contribute to a reduction in concentration by reducing the concentration of dry matter in the filtrate.

3.5 Characterization of physicochemical properties of raw juice material and retentate at a different phase of the filtration process

The physicochemical properties including total solid content (%), moisture content (%), and ash content (%) of raw juice material and retentate at different stages of processing are presented in Table 1. Generally, the ash content was found to be insignificantly different during different stages of the filtration process from raw material to the final product (p>0.05).

The difference was observed in moisture content and TSC (total solid content) values. The initial TSC value of raw material was 8.539±0.23%, which was increased to 10.43±0.07% (p<0.05). This was explained by the retention of macromolecules in the retentate, leading to the increase in TSC value. After diafiltration, the retentate was significantly reduced to 4.754±0.06 due to the addition of the water amount. It was reported that the amount of whey protein was reduced after diafiltration because of the dilution process by adding water for removing soluble molecules such as lactose and salt (Renhe *et al.,* 2019). The TSC value of the retentate after centrifugation was sharply enriched due to the removal of water after centrifugation. The TSC value of diafiltrated-centrifuged retentate was lower than centrifuged retentate which was explained by the removal of some solutes salts and sugars. The total soluble solid was found to decrease noticeably after the diafiltration process of citrus juices, as indicated by Polidori *et al.* (2018). This indicated the enhancement of lycopene content in the retentate after the diafiltration process. In terms of moisture content, the moisture content of the retentate decreased significantly after centrifugation due to the loss of water after centrifugation, from 91.461±2.31% to 50.78±3.21%, p<0.05.

Table 2 shows the difference in values of L, a, b between watermelon juice and the retentate after

Table 1. Physicochemical properties of watermelon juice material and retentate during different stages of the filtration process

	Raw juice material	Microfiltration	Diafiltrated retentate	Centrifuged retentate	Diafiltrated-centrifuged retentate
TSC (%)	8.539±0.03[b]	10.43±0.07[c]	4.754±0.06[a]	49.22±0.21[d]	42.994±0.65[c]
Moisture content (%)	91.461±2.31[b]	89.57±3.21[b]	95.246±2.32[b]	50.78±3.21[a]	57.006±4.23[a]
Ash content (%)	0.225±0.07[a]	0.182±0.03[a]	0.117±0.05[a]	0.168±0.03[a]	0.167±0.02[a]

Values with different superscripts within the same row are significantly different ($p<0.05$).

microfiltration. Both samples obtained the same lightness (L value of ~29.5) but appeared to have a difference in a* and b* values. This was due to the changes in constituents of the retentate or the increase in TSC value after microfiltration.

Table 2. Colour difference of watermelon juice and its retentate

CIE Lab colour space	L	a	b
Watermelon juice	29.51±1.3	10.08±0.42	5.21±0.22
Retentate	29.55±1.35	8.6±0.25	4.46±0.2

4. Conclusion

Factors affecting the cross-flow filtration process were investigated to obtain suitable conditions for the concentration of lycopene content in watermelon juice. Increasing the transmembrane pressure led to an increase in the permeate flux as well as the lycopene content. An extended time of filtration caused the membrane fouling, leading to a reduction in the permeate flux. The centrifugation speed and the amount of added water also affected the lycopene content. The physicochemical and nutritional properties of filtrated juice were remarkably enhanced in terms of the concentrated lycopene in the retentate. The retentate could be further utilized for developing the functional product of lycopene extract.

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