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Effect of membrane processing on biochemical quality of pomegranate and pineapple fruit juices

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Abstract
Pineapple (*Ananas comosus* L., Merril) and pomegranate (*Punica granatum* L., Punicaceae) are the most popular tropical non-citrus fruits, mainly because of their attractive aroma, refreshing flavour and favourable brix/acid ratio. The research was carried out on the biochemical analysis of membrane clarified pomegranate and pineapple juices. Membrane clarified permeates of both the juices were collected and the biochemical analysis was conducted. Among the MF membranes 0.1 µm membrane operated at 15 psi (1.0342 bar) TMP and 30 Lph flow rate gave higher flux and permeate of improved biochemical properties for pomegranate juice. In UF of pomegranate juice, in the order of permeates that recorded higher flux and improved biochemical properties were at membrane operating conditions 70 kDa, 15 psi (1.0342 bar) and 30 Lph; 44 kDa, 15 psi (1.0342 bar) and 40 Lph and 10 kDa, 15 psi (1.0342 bar) and 40 Lph. Similarly, the pineapple juice also recorded higher permeate flux and improved biochemical properties during both MF and UF when operated at 0.1 µm, 15 psi (1.0342 bar) and 30 Lph; 70 kDa, 15 psi (1.0342 bar) and 30 Lph and 44 kDa, 15 psi (1.0342 bar) and 40 Lph. It can be concluded that both MF and UF can be used to clarify juices, but UF appears to be better than MF.

Keywords: Membrane clarification, microfiltration, permeate flux, transmembrane pressure, ultrafiltration

Introduction
There is a worldwide increasing tendency for the consumption of tropical fruits, juices and fruit drinks due to the interest in ready to consume healthy products. Fruit juices are liquid foods that provide vitamins, sugars, mineral compounds and water. Consumers have individual preferences for specific appearance, consistency and flavor characteristics. Pomegranate (*Punica granatum* L., Punicaceae) and pineapple (*Ananas comosus* L., Merril) are the most popular tropical non-citrus fruits, mainly because of their attractive aroma, refreshing flavour and Brix/acid ratio. Their juices have been used in fruit based beverages individually, in the form of mixture or combined with other fruit juices. As an ingredient, the concentrated juice from pomegranate and pineapple, blends well with other aromas of fruits resulting in a pleasant product with a competitive market price. Pomegranate, mainly produced in the middle east, have a number of nutritional and health benefits and is a potential source of anthocyanins, ellagic acid, phytoestrogenic flavonoids, tannins and organic acids, some of which are antioxidants. Further, as reported in biological studies, pomegranate juice is rich in anti-atherosclerotic and anti-atherogenic compounds which have been shown to reduce blood pressure and low density lipoprotein oxidation (Aviram and Dornfeld, 2001) [2]. Pineapple juice is a popular product with increasing demand in many countries. Thailand is one of the biggest countries for the production of pineapple and exports more than a half the total of world volume annually. Pineapple juice is marketed world-wide because of its attractive aroma, flavour and other beneficial components. Nutritional compounds in pineapple juice for human health are generally identified as phytochemicals, such as vitamin C, carotenoid, flavanoid and phenolic compounds (Laorko *et al*., 2010) [11]. These components not only reduce the risk of oxidative damage related to the presence of free radicals, but also, the risk of contracting different types of cancer and cardiovascular and neurological diseases. Due to these characteristics and increasing public awareness about nutritional food, the demand for the pineapple and pomegranate fruits has

significantly increased in the last years. Consequently, many industries producing pineapple and pomegranate fruit juices as well as pharmaceutical companies extracting health beneficial compounds from the fruits have been developed.

Pineapple and pomegranate juices in its original state have a turbid appearance that makes it hard to preserve during the storage. Since the main problem with juices is stability, there is a need for research to solve this problem besides preservation of color. According to the literature, the present methods used for polyphenol elimination to produce stable juice involve liquid extraction with organic solvents. However, these methods require high temperature to increase the extraction rate and yield, but, may denature the polyphenols leading to undesirable byproducts. Therefore, MF and UF as modern methods are used to reduce juice turbidity. MF and UF are non- thermal and low cost separation technologies for juices emerging in recent years. UF and MF have been applied in vegetal juices, pulps and wine industries, reducing many steps of the conventional clarification. Also, pectinolytic enzymes can be reduced and sometimes can even be eliminated.

Purpose of this study is to evaluate the effects of clarification treatments combined with membrane filtration on organic acid compositions, phenolic compounds and on some other quality parameters including color and turbidity of pineapple and pomegranate juice. MF/UF process alone has no observable effect on the phenolic compounds and organic acid compositions of both juices. Although effects of some clarifying agents on some quality characteristics of fruit juices such as pH, turbidity, total color density, total phenolic substances and anthocyanins have been reported previously, membrane filtration has been replacing conventional treatments for clarifying fruit juices with the advantages of elimination of clarifying agents together with their associated problems and production with a continuous simplified process. By MF/UF treatment, all proteins larger than the membrane molecular weight cutoff (MWCO) can be removed.

Keeping in view of the above points, a study was undertaken on processing of pineapple and pomegranate juices that constituted the study of physicochemical characteristics of both membrane clarified juices.

Material and Methods

Fully matured pomegranate and pineapple fruits were selected and cleaned to remove extraneous material. The juices were extracted and subjected to physicochemical analysis. Pretreatment of pomegranate and pineapple was performed using a fining agent called egg albumin. The juice was subjected to four concentration levels *i.e.,* 0.25, 0.5, 1 and 2 g/L and effect of pretreatment was analysed. Among the four concentration levels, 2 g/L concentration gave good results in removal of colloidal substances of both juices. After the collection of juice, the egg albumin powder was added and mixed thoroughly. The juice samples were muslin cloth filtered and centrifuged at 4000 rpm (2147 g) for 5 min (Domingues *et al*., 2011) [9]. Colloids can decrease the permeate flux during filtration of the juice due to presence of pectinases, cellulase, hemicellulase, xylanase, carbohydrase, glucanase or arabinose. Removal of aggregates of these species via pretreatment may increase the permeate flux due to the reduction in the size of the particles and the subsequent decrease in viscosity (Valero *et al*., 2014) [21].

Membrane clarification (MF and UF) of pomegranate and pineapple juices after pretreatment was carried out in hollow fibre membrane module setup. The hollow fiber membrane set up is shown in Fig 1. Membrane processing of pomegranate and pineapple juice was carried out in the membrane module setup with different hollow fibre cartridges. The container was filled with 250 mL of juice. The operation was done in total recycle mode. The suction, retentate, by-pass lines were kept in feed solution and continuous operation was carried out. The permeate was collected at permeate line separately. All microfiltration (MF) and ultrafiltration (UF) experiments were carried out at transmembrane pressures (TMPs) of 0.3447 bar (5 psi), 0.6894 bar (10 psi), 1.0342 bar (15 psi) and 1.3789 bar (20 psi). The pore sizes of hollow fibre cartridges used for microfiltration and ultrafiltration experiments were 0.1 and 0.2 µm and 120, 70, 44 and 120 kDa (MWCO), respectively. The permeate was collected at regular intervals of time and tabulated. Initially the membranes were compacted at 1.0342 bar 15 psi, 30 Lph with distilled water for 2 hours in total recycle mode. Further, pure water flux data was collected both for MF and UF membranes using distilled water. After each run, the set up was flushed with distilled water and then cleaned with 0.1 N hydrochloric acid (HCl) for 30 mins in total recycle mode according to the washing protocol given by the manufacturer. After thorough washing, the permeability of the cartridges was analysed to measure the change in permeability of the hollow fibres. All the experiments were conducted in triplicate at room temperatures (30±2 °C). After every experiment, the membranes were cleaned properly and stored in the 1% formalin solution for future use. The permeate collected was stored in glass bottles. The experiments were performed according to the different conditions laid down in the table 1 and analysed to obtain high permeate flux.

Fig 1: Hollow fibre membrane setup

Table 1: Operating variables for microfiltration and ultrafiltration of pomegranate and pineapple juices

Operating variables	
Membrane poresizes:	MF - 0.1 and 0.2 µm UF – 120, 70, 44 and 10 kDa
Transmembrane pressures (TMP):	0.3447 bar (5 psi), 0.6894 bar (10 psi), 1.0342 bar (15 psi) and 1.3789 bar (20 psi)
Crossflow Velocities/ Feed flow rates:	0.024 m/s (20 Lph), 0.037 m/s (30 Lph) and 0.049 m/s (40 Lph)

The juices processed by different membrane pore sizes, transmembrane pressures and feed flow rates were collected and biochemical analysis was conducted.

Total soluble solids (TSS) of juice were measured by Refractometer (ATAGO make, range 58-90%) and expressed in terms of % Brix. The juice recovery per 100g of fruit was estimated. The pH measurement was performed using a digital pH meter (Systronics digital pH meter 355). The colour intensity was measured using a Systronics PC based Double Beam Spectrophotometer at absorbance of 510 nm. Similarly, Browning index was expressed as the ratio of 420 nm to 520 nm using Systronics PC based Double Beam Spectrophotometer (Valero *et al*., 2014) [21].

The turbidity and color was also measured using Systronics PC based Double Beam Spectrophotometer at absorbance of 700 nm and 420 nm respectively. The turbidity values of both juices were measured according to the procedure given by Valero *et al*. (2014) [21]. Titrable acidity of both juices are determined by the procedure of AOAC (2005) [3]. Titrable acidity is expressed as the amount of free acid mainly as anhydrous citric acid present in fruit, conveniently in g acid per 100 g or 100 ml.

$$\% \text{ acidity} = \frac{a \times b \times c \times d \times 100}{e \times w \times 1000} \quad 1$$

where, a = titre value (volume of 0.1N NaOH)
b = Normality of the alkali (0.1N)
c = volume made up
d = equivalent weight of the acid
w = weight or volume of sample taken (g or ml)
e = aliquot

Viscosity of the fruit juice was determined by using Digital Viscometer (Brookfield, Model: DV1MLV). Lane and Eynon method (Ranganna, 1986) [6] was used for determination of total, reducing and non – reducing sugars.

$$\text{Reducing sugars } \% = \frac{(\text{factor}(0.052) \times \text{dilution} \times 100)}{(\text{titre} \times \text{wt.of sample})} \quad 2$$

$$\text{Total sugars } \% = \frac{(\text{factor}(0.052) \times \text{dilution} \times 100)}{(\text{titre} \times \text{wt.of sample})} \quad 3$$

Non Reducing Sugars % = Total Sugars – Reducing Sugars (Saeed and Iftikhar *et al*., 2002, Ahmmed *et al*., 2015) [20, 1]

The antioxidant assay was estimated by ferric reducing antioxidant power method using ascorbic acid as standard and total Phenolic content by Folin Ciocalteu'smethod using gallic acid as standard (Kametkar *et al*., 2014) [10]. The total anthocyanin content was determined by the procedure given by Raj *et al*. (2011) [15]. Anthocyanins are water soluble phenolic glycosides belonged to flavonoid pigments having C_{15} skeleton of flavones as basic structural unit.

$$\text{Total O.D./100 ml} = \frac{\text{O.D.} \times \text{Volume made up} \times 100}{\text{ml of juice taken}} \quad 4$$

$$\text{Total anthocyanin (mg/100 ml)} = \frac{\text{Total O.D/100 ml}}{87.3} \quad 5$$

Results and Discussion

Development of process technology for preservation of juices involves selection of processing steps, appropriate membrane processes, membrane pore sizes and optimum operating conditions. Membrane processing was explored as an alternative to thermal/ added preservative processes or as an additional minimal processing method to achieve shelf stable high quality fruit juices. Therefore membrane processing of both pomegranate and pineapple juices was performed in combination of six different pore sizes (0.2 µm, 0.1 µm, 120 kDa, 70 kDa, 44 kDa and 10 kDa), four transmembrane pressures 0.3447, 0.6894, 1.0342 and 1.3789 bar (5, 10, 15 and 20 psi) and 3 feed flowrates (20, 30 and 40 Lph). The permeates collected from all these operational parameter combinations were analysed for biochemical quality in order to evaluate the best permeate among all the seventy two combinations. The best samples were analysed statistically using mixed anova effect.

Total soluble solids

The highest Total Soluble Solids (TSS) value of 10.322% Brix was recorded for pomegranate juice under the operational conditions of 0.1µm, 15 psi (1.0342 bar) TMP, and 30 Lph flow rate (Fig 2). Similarly, elevated TSS values were achieved with 70 kDa, 15 psi (1.0342 bar) TMP, 30 Lph (10.213%) and 44 kDa, 15 psi (1.0342 bar) TMP, 40 Lph (9.995%). These higher TSS values correlated with increased TMPs and flow rates. This trend is consistent with findings by Moreno *et al*. (2012) [12] in their research on sugarcane juice clarification. Similar observations were made for pineapple juice, where high TSS values were noted for the operational conditions of 0.1µm, 15 psi (1.0342 bar) TMP, 30 Lph (11.005%), 70 kDa, 15 psi TMP, 30 Lph (11.004%), and 44 kDa, 15 psi TMP, 40 Lph (10.994%).

The relatively higher TSS values in these permeates could be attributed to reduced fouling, allowing for almost complete permeation of solids. This phenomenon is likely to occur due to the selected operational parameters. Moreover, the comparison of permeate values with feed values indicated a significant difference ($p<0.01$), signifying the efficiency of the membrane process.

Colour intensity

The most intense color, with a value of 7.225, was observed for pineapple juice under the operational conditions of 0.1µm, 15 psi (1.0342 bar) TMP, and 30 Lph flow rate (Fig 2). Similarly, higher color intensity values were recorded with 70 kDa, 15 psi (1.0342 bar) TMP, 30 Lph (7.167) and 44 kDa, 15 psi (1.0342 bar) TMP, 40 Lph (7.153). This heightened color intensity was notably associated with elevated TMPs and flow rates. Comparable trends were found in pomegranate juice, where color intensity was high under the conditions of 0.1µm, 15 psi (1.0342 bar) TMP, 30 Lph (6.224), 70 kDa, 15 psi (1.0342 bar) TMP, 30 Lph (6.622), and 44 kDa, 15 psi (1.0342 bar) TMP, 40 Lph (6.593).

The intensified absorbance values observed in pineapple juice can be attributed to its low transmittivity. As for the color intensity values, they exhibited a decreasing trend as the membrane pore size decreased, possibly due to the limited passage of color pigments through smaller pores. These findings align with the outcomes observed by Mirsaeedghazi *et al*. (2010) [13] during their research on the clarification of pomegranate juice.

Browning index (BI)

The operational configuration of 10 kDa, 15 psi (1.0342 bar) TMP, and 40 Lph flow rate for pomegranate juice resulted in the lowest browning index, with a value of 3.722 (Fig 2). Similarly, lower browning index values were obtained with 70 kDa, 15 psi (1.0342 bar) TMP, 30 Lph (3.768) and 44 kDa, 15 psi (1.0342 bar) TMP, 40 Lph (3.746). Comparable trends were observed for pineapple juice, with the lowest browning index values recorded under the conditions of 44 kDa, 15 psi (1.0342 bar) TMP, 40 Lph (3.687); 70 kDa, 15 psi (1.0342 bar) TMP, 30 Lph (3.864); and 0.1µm, 15 psi (1.0342 bar) TMP, 30 Lph (5.126).

Notably, pomegranate juice exhibited lower browning index values compared to pineapple juice. This decrease in browning index was consistent with the decrease in membrane pore size. These findings resonate with the research conducted by Valero *et al*. (2014) [21], who observed similar trends when clarifying pomegranate juice using egg albumin as a clarifying agent.

Turbidity

The lowest recorded turbidity value of 0.421 was observed for the operational condition of 10 kDa, 15 psi (1.0342 bar) TMP, and 40 Lph flow rate for pomegranate juice (Fig 2). Likewise, other notably low turbidity values were obtained: 0.422, 0.423, and 0.465 for 44 kDa, 15 psi (1.0342 bar) TMP, 40 Lph; 70 kDa, 15 psi (1.0342 bar) TMP, 30 Lph; and 0.1µm, 15 psi (1.0342 bar) TMP, 30 Lph, respectively. The same trend was observed for pineapple juice, with the lowest turbidity levels being achieved under the conditions of 44 kDa, 15 psi (1.0342 bar) TMP, 40 Lph; 70 kDa, 15 psi (1.0342 bar) TMP, 30 Lph; and 0.1µm, 15 psi (1.0342 bar) TMP, 30 Lph, recorded as 0.485, 0.486, and 0.579, respectively.

This decrease in turbidity for both pomegranate and pineapple juices can be attributed to the reduction in total soluble solids. These findings align with similar observations made by Mirsaeedghazi *et al*. (2010) [13] and Carvalho *et al*. (1998) [8], demonstrating the consistent impact of membrane processing on reducing turbidity levels in fruit juices.

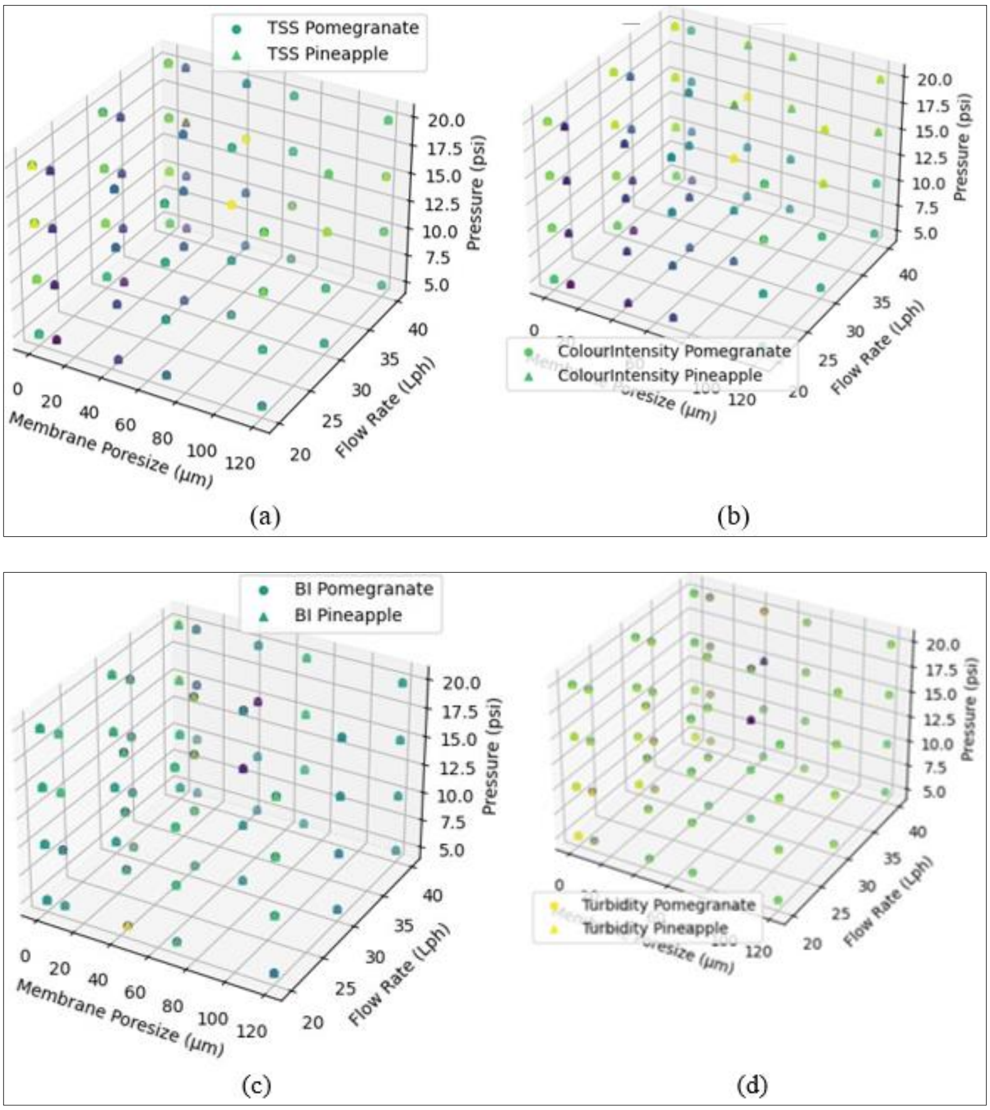

Fig 2: Variation of different bio-chemical attributes upon membrane clarification for both pomegranate and pineapple juices (a) TSS (b) Colour intensity (c) Browning index (BI) (d) Turbidity

Titrable Acidity

For pomegranate juice, the titrable acidity values were found to be 0.756%, 0.755%, 0.738%, and 0.734% for the permeates of 0.1µm, 15 psi (1.0342 bar) TMP, 30 Lph; 70 kDa, 15 psi (1.0342 bar) TMP, 30 Lph; 44 kDa, 15 psi (1.0342 bar) TMP, 40 Lph; and 10 kDa, 15 psi (1.0342 bar) TMP, 40 Lph, respectively (Fig 3). Similarly, the titrable acidity values for pineapple juice permeates were measured as 1.925%, 1.874%, and 1.785% for the respective operational conditions of 0.1µm, 15 psi (1.0342 bar) TMP, 30 Lph; 70 kDa, 15 psi (1.0342 bar) TMP, 30 Lph; and 44 kDa, 15 psi (1.0342 bar) TMP, 40 Lph.

The data clearly indicates that pineapple juice has a higher titrable acidity compared to pomegranate juice. Moreover, the results suggest that as the membrane pore size decreases, the titrable acidity tends to decrease as well, which is consistent with findings from previous research by Carvalho *et al*. (1998) [8].

Furthermore, an increase in titrable acidity was observed with higher values of TMPs and flow rates. Interestingly, this is contrary to the study conducted by Couto *et al*. (2011) [5], where the concentration process of pineapple juice led to a 2.9-fold increase in titrable acidity. In our study, which focused on the clarification process using various membranes, a decrease in titrable acidity was observed.

Lastly, it's worth noting that the permeate values were compared with the initial feed values, and a significant difference ($p<0.01$) was observed, highlighting the impact of the membrane processing on the titrable acidity levels of both juices.

pH

The lowest pH values obtained for pomegranate juice were 4.550, 4.567, 4.58, and 4.59 under the following conditions: 0.1µm Membrane Pore Size, 15 psi (1.0342 bar) Transmembrane Pressure (TMP), and 30 Lph flow rate; 70 kDa MWCO, 15 psi TMP, and 30 Lph flow rate; 44 kDa MWCO, 15 psi TMP, and 40 Lph flow rate; and 10 kDa MWCO, 15 psi TMP, and 40 Lph flow rate, respectively (Fig 3). Similarly, the pH values for pineapple juice were recorded as 4.56, 4.647, and 4.654 under the conditions of 0.1µm Membrane Pore Size, 15 psi TMP, and 30 Lph flow rate; 70 kDa MWCO, 15 psi TMP, and 30 Lph flow rate; and 44 kDa MWCO, 15 psi TMP, and 40 Lph flow rate, respectively.

There exists an inverse relationship between the values of titrable acidity and pH. As observed, the titrable acidity values decreased during the membrane processing, likely due to the reduction of acidic components. Consequently, this reduction in acidity contributed to an increase in the pH values. It's notable that the permeate values were compared with the initial feed values, revealing a significant difference ($p<0.01$) between them.

The obtained permeates for both pomegranate and pineapple juices exhibited the desired low pH values, meeting the quality standards for juices. Additionally, when considering the combined effects of different operational parameters, there was a notable and statistically significant difference ($p<0.05$) observed among the pH values of all the permeates for both types of juices. This emphasizes the influence of the processing conditions on the resulting pH levels.

Relevantly, the research conducted by Couto *et al*. (2011) [5] revealed that the pH values of juices remained relatively stable during the concentration process achieved through reverse osmosis. This aligns with our findings, suggesting that the chosen membrane processing conditions have a limited impact on the pH values of the juices

Viscosity

The most minimal viscosity values recorded for pomegranate juice were 1.81 cP, 1.67 cP, 1.3 cP, and 1.28 cP, achieved under the conditions of 70 kDa MWCO, 15 psi (1.0342 bar) Transmembrane Pressure (TMP), and 30 Lph flow rate; 44 kDa MWCO, 15 psi TMP, and 40 Lph flow rate; and 10 kDa MWCO, 15 psi TMP, and 40 Lph flow rate, respectively (Fig 3). Similarly, the viscosity values for pineapple juice were 1.973 cP, 1.257 cP, and 1.247 cP for the conditions of 0.1µm Membrane Pore Size, 15 psi TMP, and 30 Lph flow rate; 70 kDa MWCO, 15 psi TMP, and 30 Lph flow rate; and 44 kDa MWCO, 15 psi TMP, and 40 Lph flow rate, respectively.

Comparatively, the viscosity of pineapple juice was higher than that of pomegranate juice. This discrepancy in viscosity can be attributed to the variations in compositional attributes of the two juices. This observation is consistent with the findings of Carneiro *et al*. (2002) [6], who reported a decrease in viscosity by 29.6% for single-strength pineapple juice to hydrolyzed juice and a reduction of 22.0% for pulp content. The decrease in viscosity with decreasing membrane pore size is attributed to the restriction of larger colloidal components from passing through the membrane, consequently resulting in lowered viscosity.

Total antioxidant activity

The pomegranate juice permeates with high Total Anthocyanin Content (TAA) were obtained under the following conditions: 0.1µm Membrane Pore Size, 15 psi (1.0342 bar) Transmembrane Pressure (TMP), and a Flow Rate of 30 Lph (30.572 mg/g); 70 kDa MWCO, 15 psi TMP, 30 Lph (30.512 mg/g); and 44 kDa MWCO, 15 psi TMP, and 40 Lph (30.412 mg/g) (Fig 3). Similarly, the permeate samples with elevated TAA values for pineapple juice were obtained with the same conditions: 0.1µm Membrane Pore Size, 15 psi TMP, 30 Lph (12.6333 mg/g); 70 kDa MWCO, 15 psi TMP, 30 Lph (12.4333 mg/g); and 44 kDa MWCO, 15 psi TMP, and 40 Lph (8.7132 mg/g).

Clearly, the data indicated that the Total Anthocyanin Content was higher for pomegranate juice than for pineapple juice. Furthermore, it was observed that a decrease in pore size corresponded to a reduction in TAA for both juices. This trend aligns with the findings of Mirsaeedghazi *et al*. (2010) during the clarification of pomegranate juice, suggesting that changes in membrane pore size can influence the anthocyanin content.

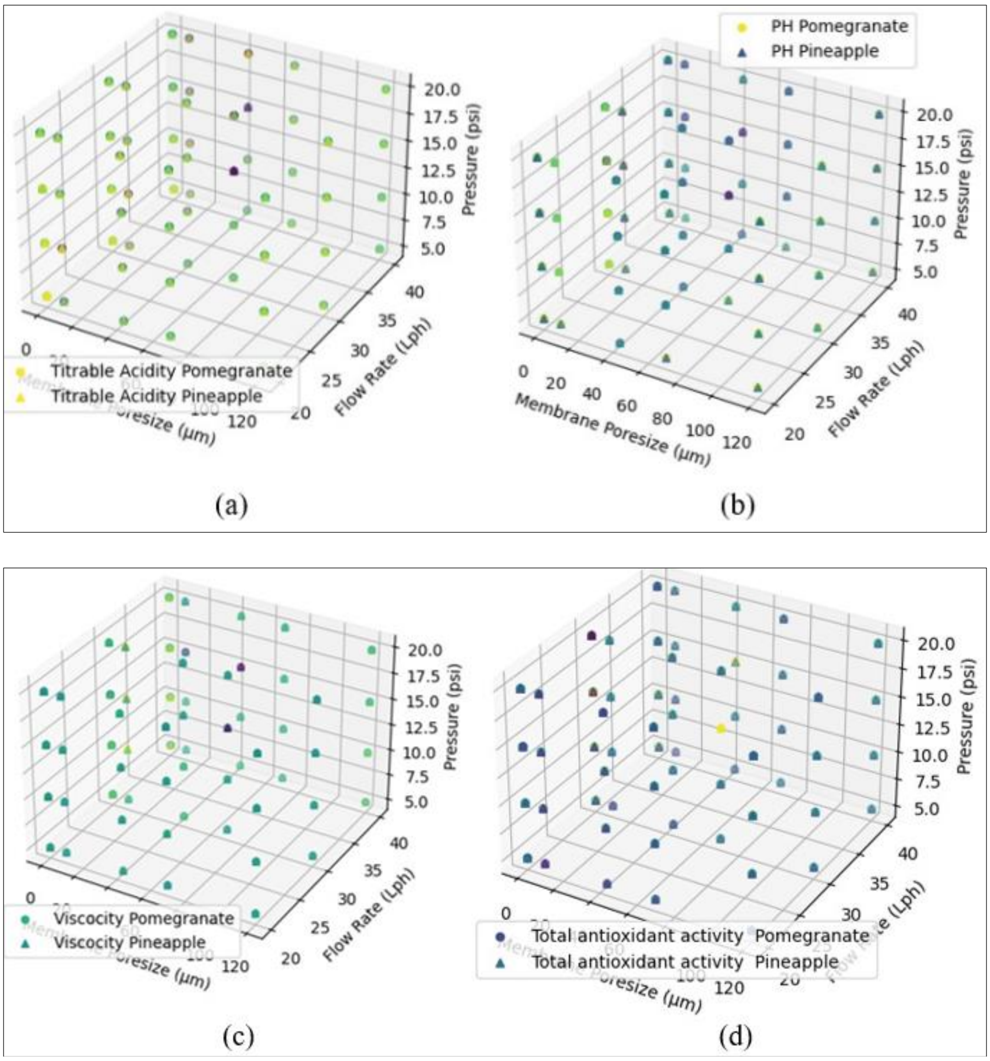

Fig 3: Variation of different bio-chemical attributes upon membrane clarification for both pomegranate and pineapple juices (a) Titrable acidity (b) pH (c) Viscosity (d) Total antioxidant activity

Total phenolic content

The operational parameter combinations that yielded high Total Phenolic Content (TPC) for pomegranate juice were as follows: 0.1µm Membrane Pore Size, 15 psi (1.0342 bar) Transmembrane Pressure (TMP), and a Flow Rate of 30 Lph (53.004 mg of GAE/g of dry material); 70 kDa MWCO, 15 psi TMP, 30 Lph (52.334 mg of GAE/g); 44 kDa MWCO, 15 psi TMP, and 40 Lph (52.224 mg of GAE/g); and 10 kDa MWCO, 15 psi TMP, and 40 Lph (51.752 mg of GAE/g) (Fig 4). Similarly, the operational parameters that yielded high TPC for pineapple juice were: 0.1µm Membrane Pore Size, 15 psi TMP, 30 Lph (59.245 mg of GAE/g); 70 kDa MWCO, 15 psi TMP, 30 Lph (58.227 mg of GAE/g); and 44 kDa MWCO, 15 psi TMP, and 40 Lph (58.219 mg of GAE/g).

From the data, it's evident that pineapple juice exhibited a higher phenolic content compared to pomegranate juice. Notably, significant differences ($p<0.05$) were observed among the TPC values of all the permeate juices. This decrease in phenolic content as the pore size decreased could be attributed to the potential retention of certain components. Wen and Wrolstad (2002) [22] reported the presence of non-flavonoid phenolics in pineapple fruit, juice, and shell fibers. Couto *et al*. (2011) [5] demonstrated that concentrating pineapple juice through reverse osmosis led to increased phenolic compounds, indicating a 2.6-fold increase in phenolic content in the retentate side. Comparing the permeate values with the initial feed values revealed a significant distinction ($p<0.01$), underscoring the impact of membrane processing on phenolic content.

Total anthocyanin content

Permeate samples from pomegranate juice displaying elevated Total Antioxidant Capacity (TAC) levels were observed under specific conditions: 0.1µm Membrane Pore Size, 15 psi (1.0342 bar) Transmembrane Pressure (TMP), and a Flow Rate of 30 Lph (10.016 mg/100 mL); 70 kDa MWCO, 15 psi TMP, 30 Lph (10.013 mg/100 mL); 44 kDa MWCO, 15 psi TMP, and 40 Lph (10.008 mg/100 mL); and 10 kDa MWCO, 15 psi TMP, and 40 Lph (9.896 mg/100 mL) (Fig 4). Correspondingly, the permeate samples exhibiting elevated TAC levels in pineapple juice were observed under the conditions of: 0.1µm Membrane Pore Size, 15 psi TMP, 30 Lph (0.5846 mg/100 mL); 70 kDa MWCO, 15 psi TMP, 30 Lph (0.5935 mg/100 mL); and 44 kDa MWCO, 15 psi TMP, and 40 Lph (0.5927 mg/100 mL). It's noteworthy that a reduction in anthocyanin content was observed as the membrane pore size decreased. Moreover, the highest anthocyanin levels were achieved at elevated TMPs and flow rates for both pineapple and pomegranate juices. Importantly, pomegranate juice exhibited significantly higher anthocyanin contents compared to pineapple juice. The permeate values were subjected to comparison with the initial feed values, revealing a significant discrepancy ($p<0.01$) between the two datasets. This emphasizes the tangible influence of the membrane processing on the anthocyanin content of both juices.

Reducing sugars

The samples from the permeate with elevated levels of reducing sugars were subjected to analysis for both pomegranate and pineapple juices. For pineapple juice, the permeates exhibiting notable recovery of reducing sugars were observed under the following conditions: 0.1 µm membrane pore size, 15 psi (1.0342 bar) transmembrane pressure (TMP), and a flow rate of 30 Lph; 70 kDa membrane pore size, 15 psi (1.0342 bar) TMP, and a flow rate of 30 Lph; and 44 kDa membrane pore size, 15 psi (1.0342 bar) TMP, and a flow rate of 40 Lph. The percentages of recovery were 7.497%, 6.847%, and 6.636%, respectively (Fig 4).

Similarly, for pomegranate juice, the permeate samples demonstrating substantial reducing sugars were identified under the following conditions: 0.1 µm membrane pore size, 15 psi (1.0342 bar) TMP, and a flow rate of 30 Lph; 70 kDa membrane pore size, 15 psi (1.0342 bar) TMP, and a flow rate of 30 Lph; 44 kDa membrane pore size, 15 psi (1.0342 bar) TMP, and a flow rate of 40 Lph; and 10 kDa membrane pore size, 15 psi (1.0342 bar) TMP, and a flow rate of 40 Lph. The recovery percentages were 4.992%, 4.987%, 4.985%, and 4.968%, respectively.

Comparatively, pineapple juice exhibited higher levels of reducing sugars in contrast to pomegranate juice. Elevated reducing sugar values were achieved at increased TMPs and flow rates. It was discerned that both juices displayed a reduction in reducing sugars corresponding to larger membrane pore sizes. A study by Carvalho *et al*. (2008) [4] also revealed the loss of sugars and soluble solids due to factors like membrane cut-off, configuration, temperature, and the presence of concentration polarization and fouling. These findings align with our study, demonstrating a decline in sugars. A notable statistical distinction ($p<0.05$) emerged among the combinations of treatments for both juices. Moreover, a significant variance ($p<0.01$) with feed values was observed when comparing the permeate values.

Non- reducing sugars

Notable levels of non-reducing sugars were documented for pomegranate juice permeates obtained under specific conditions, including: 0.1µm Membrane Pore Size, 15 psi (1.0342 bar) Transmembrane Pressure (TMP), and a Flow Rate of 30 Lph; 70 kDa MWCO, 15 psi TMP, 30 Lph; 44 kDa MWCO, 15 psi TMP, and 40 Lph; and 10 kDa MWCO, 15 psi TMP, and 40 Lph. The recorded non-reducing sugar percentages for these permeates were 3.024%, 3.025%, 3.002%, and 1.958%, respectively (Fig 4). Similarly, for pineapple juice, the non-reducing sugar percentages for the corresponding permeates were: 0.1µm Membrane Pore Size, 15 psi TMP, and a Flow Rate of 30 Lph (1.355%); 70 kDa MWCO, 15 psi TMP, 30 Lph (2.001%); and 44 kDa MWCO, 15 psi TMP, and 40 Lph (2.189%).

It's noteworthy that the pomegranate juice permeates demonstrated higher non-reducing sugar content compared to the pineapple juice permeates. Moreover, a thorough comparison was conducted between the permeate values and the initial feed values. This analysis highlighted a significant distinction ($p<0.01$) between the obtained permeate values and the original feed values. This indicates that the membrane separation process had a discernible impact on the non-reducing sugar composition of both pomegranate and pineapple juice permeates.

Total sugars

The pomegranate juice permeates that exhibited a notable recovery of total sugars were obtained under specific processing conditions. For instance, the conditions of 0.1µm Membrane Pore Size, 15 psi (1.0342 bar) Transmembrane Pressure (TMP), and a Flow Rate of 30 Lph; 70 kDa MWCO, 15 psi TMP, 30 Lph; 44 kDa MWCO, 15 psi TMP, and 40 Lph; and 10 kDa MWCO, 15 psi TMP, and 40 Lph, resulted in total sugar recoveries of 8.016%, 8.012%, 7.987%, and 6.926%, respectively (Fig 4). Similarly, for pineapple juice, high total sugar recovery was observed under the same conditions: 0.1µm Membrane Pore Size, 15 psi TMP, and a Flow Rate of 30 Lph; 70 kDa MWCO, 15 psi TMP, 30 Lph; and 44 kDa MWCO, 15 psi TMP, and 40 Lph, yielding total sugar recoveries of 8.852%, 8.848%, and 8.825%, respectively.

It's worth noting that pineapple juice exhibited higher total sugar content compared to pomegranate juice. This trend in total sugars was consistent with the patterns observed for reducing sugars. The findings of Carvalho *et al*. (2008) [4] corroborate these observations, as they reported the retention of sugars and soluble solids when using membranes with smaller cut-off sizes and specific configurations. Additionally, their study highlighted that higher Transmembrane Pressures (TMPs) were associated with increased sugar recoveries in the permeate.

Furthermore, the research by Oliveria *et al*. (2012) [14] emphasized that higher TMPs lead to significant impediment to sugar passage across the membrane, resulting in a reduction in total sugar content in the permeate. These studies collectively underline the critical role of membrane cut-off sizes and TMPs in influencing sugar recovery during membrane processing. The choice of membrane characteristics, coupled with appropriate processing conditions, has a direct impact on the separation and retention of total sugars in fruit juice permeates, as evident from both the pomegranate and pineapple juice data.

Colour

Based on the provided data, it is evident that both pomegranate and pineapple juice samples with high color values were subjected to specific membrane processing conditions. For pomegranate juice, the permeate samples with high color values were obtained under the following conditions: 0.1µm Membrane Pore Size, 15 psi (1.0342 bar) TMP, and a Flow Rate of 30 Lph, for the 70 kDa, 44 kDa, and 10 kDa molecular weight cut-off (MWCO) membranes, resulting in color values of 3.0856, 3.0146, 3.0115, and 2.9448, respectively (Fig 4). Similarly, for pineapple juice, the high color values were observed for the same processing conditions with 0.1µm Membrane Pore Size, 15 psi (1.0342 bar) TMP, and a Flow Rate of 30 Lph, for the 70 kDa and 44 kDa MWCO membranes, resulting in color values of 7.025, 7.015, and 7.014, respectively.

The color values for both pomegranate and pineapple juices were significantly reduced under these specific membrane processing conditions. This reduction in color values is likely attributed to the decrease in anthocyanin content. Anthocyanins are color pigments present in fruits responsible for their vibrant hues, and their concentration tends to decrease as the membrane pore size is reduced. As a result, when using smaller membrane pore sizes (such as 0.1µm), more anthocyanins are retained, leading to a higher color intensity in the permeate.

The study's observations align with findings reported by Oliveiria *et al*. (2012) [14], suggesting that membrane processing with smaller pore sizes results in a decrease in color values due to the retention of color pigments, including anthocyanins. This finding underscores the importance of membrane selection in fruit juice processing to achieve desired color characteristics. By carefully choosing the appropriate membrane pore size and MWCO, fruit juice manufacturers can control the color attributes of their products and produce juices with consistent color quality.

Overall, the data demonstrates the impact of membrane processing on the color of pomegranate and pineapple juices and highlights the significance of membrane properties in determining the color values in fruit juice permeates. These insights can assist juice manufacturers in optimizing their processes to achieve desired color profiles and maintain product quality.

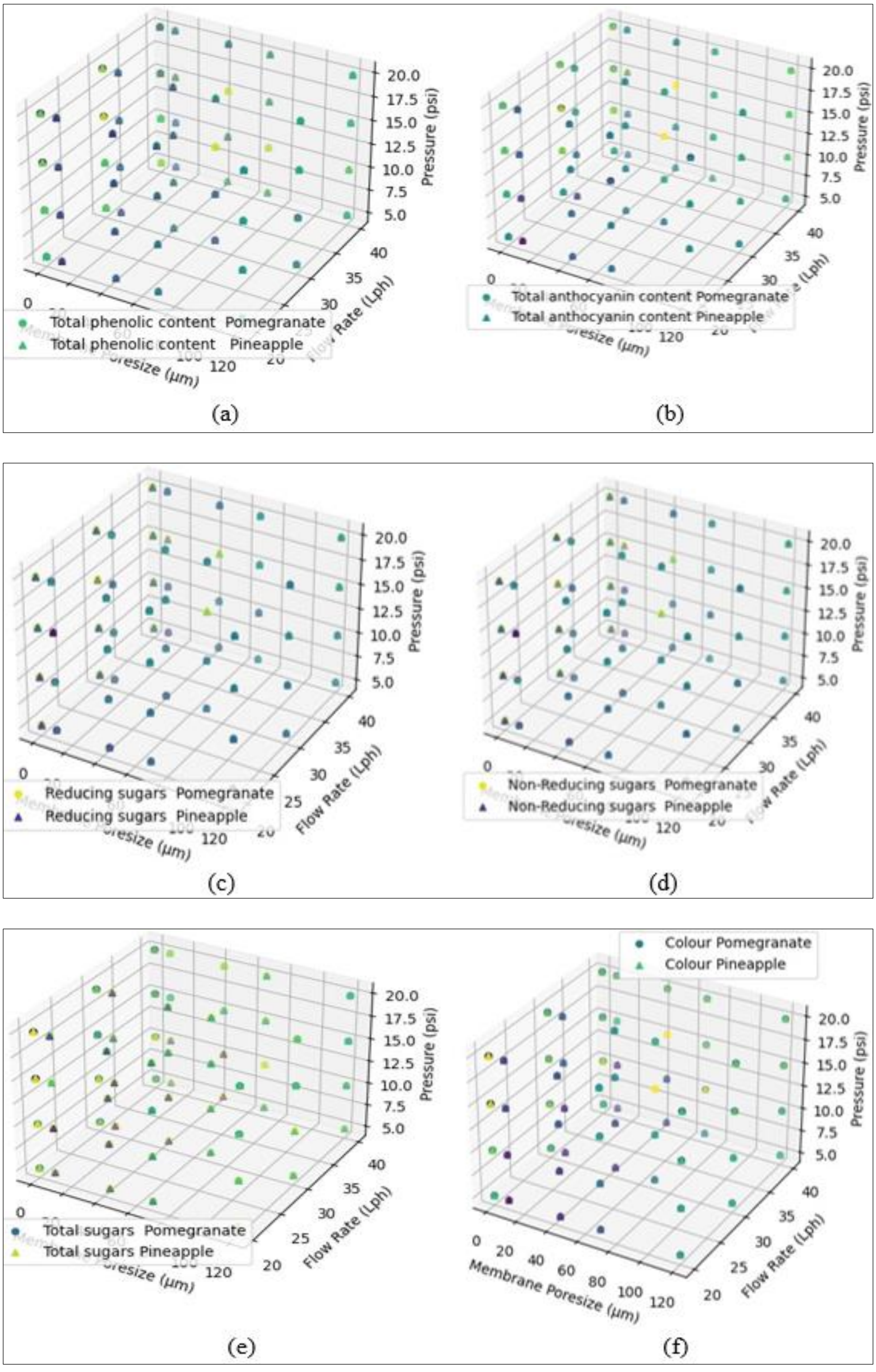

Fig 4: Variation of different bio-chemical attributes upon membrane clarification for both pomegranate and pineapple juices (a) Total phenolic content (b) Total anthocyanin content (c) Reducing sugars (d) Non- reducing sugars (e) Total sugars (f) Colour

Conclusions

Permeates from both juices were collected after membrane clarification, followed by conducting biochemical analysis. For pomegranate juice, using a 0.1 µm membrane with a transmembrane pressure (TMP) of 15 psi (1.0342 bar) and a flow rate of 30 Lph resulted in higher flux and improved biochemical properties in the permeate when using microfiltration (MF) membranes. In ultrafiltration (UF) of pomegranate juice, treatments with membranes of 70 kDa at 15 psi (1.0342 bar) and 30 Lph, 44 kDa at 15 psi (1.0342 bar) and 40 Lph, and 10 kDa at 15 psi (1.0342 bar) and 40 Lph recorded increased flux and enhanced biochemical properties in the permeate.

Similarly, for pineapple juice, the treatments that achieved higher permeate flux and improved biochemical properties using both MF and UF were as follows: 0.1 µm at 15 psi (1.0342 bar) and 30 Lph, 70 kDa at 15 psi (1.0342 bar) and 30 Lph, and 44 kDa at 15 psi (1.0342 bar) and 40 Lph.

In conclusion, both microfiltration (MF) and ultrafiltration (UF) can be effectively employed for juice clarification. However, UF appears to offer better results than MF in terms of permeate quality and flux."

Author Contributions

Study conception and design: Samreen; Bibliographic research and data interpretation: Samreen, Veera Venkata Satyanarayana Chilukuri, Edukondalu Lingathoti, Vimala Beera and Srinivasa Rao Vatluri; Critical review of the manuscript: Samreen, Veera Venkata Satyanarayana Chilukuri

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