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Valorization of pomegranate husk – Integration of extraction with nanofiltration for concentrated polyphenols recovery

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Abstract

A process development study is reported, involving water-based extraction of polyphenols from pomegranate husk and their subsequent membrane concentration. Aiming to optimize extraction, various experimental conditions were investigated, including extraction duration, solid to liquid ratio (2-14 % w/v dry weight), extraction temperature, number of sequential extraction stages (single, double and triple) and type of solvent. It was found that two extraction stages (90 min each) under a solid to water ratio of 3% w/v at 30ºC, were sufficient for obtaining a yield of 85% of total polyphenols from the husk. Subsequently, nanofiltration (NF) was used to concentrate the polyphenols extracts by a volume concentration factor (VCF) of three. Two main process parameters, namely pressure (4-14bar) and pH (4-8), were examined at constant temperature 30◦C, in batch concentration mode. Results show near optimum NF-membrane performance, with high total polyphenols retention (≥ 98%) at 10 bar pressure and pH 6. Interestingly, the NF-membrane, used for up to 10 filtration batches, exhibited satisfactory flux without intermediate cleaning, suggesting that it could be employed for an extended period. Prospects and challenges are discussed for further process development and practical application.

Keywords: pomegranate phytochemicals, agri-food wastes valorization, aqueous extraction, polyphenols nanofiltration, polyphenols concentrate

1. Introduction

Pomegranate (*Punica granatum L.*) is considered among the first edible fruits cultivated on earth [1-4] and has been used through history as folk medicine of different civilizations [1, 5-7]. Pomegranate was native to the Middle East and was later cultivated in the Mediterranean region, growing easily in semi-arid, mild-temperate to subtropical climates, such as Iran, India, China, Spain, Greece etc. [3, 4, 6]. Pomegranate consumption has increased substantially, especially as juice, in recent decades due to its positive effects on human health, mainly attributed to its anti-oxidant [1, 4, 8-10] and antimicrobial [7] properties.

The agri-food processing industries generate large amounts of waste material, which have emerged as an ideal source for extraction of bioactive compounds. The development of efficient downstream processes for their recovery and purification would provide a strong economic incentive for the agri-food sector to pursue its exploitation, with a direct positive environmental impact. Pomegranate husk comprises more than 40% [1-3, 6-12] of the whole fruit wet weight, and currently is mostly disposed of as waste, leading to various environmental issues. However, fruits exocarps (peels, husks, rinds, etc.), in general, exhibit higher anti-oxidant activity than the pulp. The pomegranate is a good example of such fruit [1, 5, 8] with its husk rich in polyphenols, especially in ellagitannins, which are ellagic acid derivatives, (punicalagin and punicalin [1, 3, 6, 12] molecular weight, MW, 1084 and 783 Da respectively), hydroxybenzoic acids [3, 11, 12] (average MW of the monomers ≥ 170 Da) and flavonoids [6] (average MW of the monomers ≥ 250 Da). Punicalagin is the main phenolic compound of the husk with remarkable biological activities [1, 13]. Thus, pomegranate husk is an inexpensive and abundant source of these polyphenols [1, 14], which could be used additionally for ellagic acid production [14].

To obtain a potentially marketable polyphenols concentrate from pomegranate solid-waste/husk, a sustainable and cost-effective process is required, comprised of two main

operations; i.e. polyphenol extraction and extract concentration. These sequential operations should be well integrated, regarding their design and operating conditions that would optimize the overall process. The study reported herein is motivated by the need to develop such an integrated process of practical applicability.

Water, as an environment friendly solvent, deserves particular attention for antioxidants extraction from pomegranate husk; therefore, it is used as the main extraction solvent in this research. In polyphenols extraction by water, temperature is a major process parameter that should be optimized. On one hand, sufficiently high temperature tends to improve the solvation power on polyphenols; on the other, at elevated temperatures, certain polyphenols are prone to degradation (particularly under prolonged extraction times) and energy consumption is increased. The literature is considered deficient on these issues, despite some work on aqueous extraction of polyphenols from pomegranate husk [1, 15-17]. Extraction methods using pressurized water [1], or water coupled with ultrasounds [12], appear to have higher cost and require special equipment. In addition to temperature, other key extraction parameters need to be optimized [16], including water/solvent quality and quantity.

Despite the advantages of water as an agent for selective extraction of antioxidants and a non-toxic solvent for processing nutraceuticals and food ingredients, its low solvation capacity leads to relatively large volumes (for achieving a satisfactory recovery yield) which negatively impact on process sustainability; therefore, other solvents are also considered, with improved solvation capacity. Water/ethanol mixtures have been already successfully employed for the extraction of several classes of polyphenols [17]. Unlike other solvents, such as methanol or acetone, ethanol is less toxic and can be reused following its recovery by distillation, thus reducing its environmental impact. In addition, ethanol is a bio-solvent produced via fermentation from various raw carbohydrate-containing materials. For these

reasons, ethanol-water mixtures have been also tested in this study in respect of their polyphenol-extraction efficiency.

Concentration of the rather dilute aqueous polyphenol extracts, to a level sufficient for direct marketing or further separation of specific compounds, is a second important process step. Selection of appropriate technology for this step should be based on usual sustainability criteria (environmental, economic) under the constraint of mild processing conditions to avoid polyphenols degradation. Obviously, conserving water/solvent and energy are prime targets in such selection. Membrane processes have emerged in recent years as strong candidates for the aforementioned tasks, due to their energy efficiency, mild processing conditions as well as facile process design and operation.

Indeed, membrane technology appears to be most appropriate for concentrating and/or selectively fractionating bioactive compounds from aqueous, or low in ethanol, processing streams such as products, by-products and wastes from agri-food industry. Reported successful applications include the concentration [18, 19] and fractionation [20, 21] of grape polyphenols; the fractionation of polyphenols from mushrooms [22], persimmon pulp [23], almond skin extracts [24], mulberry root cortices [25], black [26] and green tea [27], olive polyphenols [28] and *Salvia miltiorrhiza* [29]. The nanofiltration (NF) membrane operation, investigated herein for polyphenols concentration, is selected on the basis of past experience [19]; NF is also promising in respect of permeate quality that would allow water/solvent recycling.

NF is based on both size exclusion and charge effect. The pH of aqueous solution appears to affect the chemistry of both the compounds to be separated and the polymeric membrane surface [30, 31]. The membrane surface charge depends on the pH of the solution in contact [30]. This charge influences the distribution of ions and polar compounds at the membrane-solution interface. Ionised species of the same charge are repelled from the surface while oppositely charged species are attracted to it [30]. Electrostatic repulsion and attraction are

used in literature to explain the observed phenomenon related to flux/permeability, solute rejection and fouling behaviour [32]. The polyphenols ionization in different pH appears to play an important role for their membrane recovery. The polyphenols usually have in their molecules carboxylic acid and hydroxyl moieties. The carboxyl groups are usually in their ionic form at pH above 4, whereas the hydroxyl species are ionized at higher pH. To the best of authors knowledge, the effect of pH in polyphenols recovery by NF has been examined only in a recent study with a model mixture [28]. The effect of pH on NF performance is quite complicated, as the surface charge and the solute charge depend on membrane material and solute type [30, 31]. Polymeric NF membranes typically exhibit different surface charges in the usual pH range of 3-8 [31], therefore pH is another process parameter examined in this study.

In the following, materials and methods employed are outlined, regarding the pomegranate waste/solid characterization, extraction and membrane concentration. The respective experimental results are presented next, and an identified narrow range of near optimum conditions is discussed. Concluding remarks and prospects for practical applications follow.

2. Materials and methods

2.1. Materials

Ethanol, Na_2CO_3, $NaNO_2$, $AlCl_3$, HCl, KIO_3, NaOH were of analytical grade, purchased from Sigma-Aldrich.

Three samples of approximately 10 kg of pomegranate fruits, collected during the months October to November from northern Greece (Drama region), was used in this study. Injured fruits were discarded. After peeling the fruits and separating the arils, the weight of pomegranate wastes (husk and pith-carpellary membrane) were determined. **Table 1** includes several physical and chemical characteristics, for the different parts of the wastes, discussed in the following,

2.2. Pomegranate waste characterization

For moisture determination, 5 g mass from each waste was dried at 105^{o}C for 24h until stable weight is achieved. For the total polyphenols quantification, the wet husk and pith-carpellary membrane was homogenized with a home blender for 15 min and directly analyzed without drying to avoid polyphenols degradation. The determination of the maximum amount of total polyphenols, flavonoids and hydrolysable tannins in the homogenized waste (after the supernatant liquid of 1st extraction step was removed) was treated sequentially several times with deionized water for 90 min each time, under a constant ratio 3% w/v in dry weight basis, until none of them could be measured in the supernatant according to the methodology described below. The cumulative amount recovered from all the above described extraction steps is considered as the maximum compound content in the pomegranate wastes.

2.3. Analysis of the extracts

2.3.1. Total polyphenols concentration

Total polyphenol content (TPC) was determined as tannic acid equivalent (TAE) at 750 nm with the Folin-Ciocalteau method described by Box [33] after appropriate modification. Briefly, 150 μL Na_2CO_3 was added into 1 mL of appropriately diluted polyphenols extract, followed by 50 μL of Folin-Ciocalteau reagent and left for 1 h in room temperature to react in absence of light. The absorbance of the blue colored mixture was read at 750 nm versus the prepared blank with water containing the rest of reagents. Six different concentrations of tannic acid solutions (20–100 mg/L) were used for calibrations. The final results were expressed as mg tannic acid equivalent (TPC-TAE) per g of dry matter. This method was employed to monitor variations in the concentrations of total polyphenols in the extracts, rather than to determine the individual polyphenols absolute concentrations. This is considered sufficient for the present process development study.

2.2.2. Total flavonoid concentration

Total flavonoid concentration (TFC) of the extracts was determined by the method of Zhishen et al. [34]. Briefly, 1 mL of diluted extract was added into a 15 mL test tube containing 4 mL of water. At zero time, 0.3 mL of 5% $NaNO_2$ was added to the tube. After 5 min, 0.3 mL of 10% $AlCl_3$ was added into the tube. At 6 min, 2 mL of 1 M NaOH was added to the mixture. Immediately thereafter, the reaction tube was diluted to final volume of 10 mL with the addition of 2.4 mL of distilled water and thoroughly mixed. Absorbance of the pink colored mixture was read at 510 nm versus the prepared water blank. Six different concentrations of catechin solutions (20–100 mg/L) were used for calibrations. The final results were expressed as mg catechin equivalent (TFC-CE) per g of dry matter.

2.2.3. Hydrolyzable tannins

Hydrolyzable tannins (HT) were determined by the method of Willis and Allen [35] with slight modifications. One mL of 10-fold diluted extracts and 5 mL of 2.5% KIO_3 were added into a vial and vortexed for 10 s. In preliminary experiments (data not shown), optimum reaction time to gain maximum absorbance value was determined to be 2 min for pomegranate husk extracts and 4 min for standard solutions of tannic acid. Absorbance of the red colored mixture was determined at 550 nm versus the prepared water blank. Six different concentrations of tannic acid solutions (70–1100 mg/L) were used for calibrations. The final results were expressed as mg tannic acid equivalent (TAE-HT) per g of dry matter.

2.3 Study of the extraction conditions

For the pomegranate husk total polyphenols recovery, four parameters affecting their extraction were studied, namely extraction duration, solids to solvent ratio (in equivalent dry basis taking into account the moisture), temperature and different solvents (deionized and tap water, recovered NF permeate and mixtures of water with ethanol).

For investigating the extraction duration from pomegranate husk, 1 g of homogenized pomegranate husk was weighed into a screw-capped glass vial and a solids (basis equivalent dry mass) to solvent ratio at 3 % w/v was used, if not otherwise denoted. A magnetic stirrer bar was placed into each vial. A 100 μL sample was taken at times 2, 5, 8, 10, 12, 15, 20, 30, 40, 50, 60, 120, 180, 240 and 300 min, diluted appropriately in each case to be within the calibration range, filtered by 0.45μm membrane syringe filter and stored at -20ºC until analyzed for TPC-TAE, HT-TAE and TFC-CE content.

To examine the effect of solid-to-liquid ratio, 1 g of homogenized pomegranate husk was weighed into a screw-capped glass vial, at 25 °C and water was added to adjust the solid to solvent ratio at 2, 3, 5, 7, 9, 11 and 14 % w/v in dry basis equivalent. TPC-TAE. To study the effect of temperature in the total polyphenols extraction, a solids to solvent ratio 3% w/v was used under 14, 35, 45 and 55ºC. TPC-TAE was measured after 300 min extraction.

In addition to deionized water, tap water, ethanol-water mixtures (10 and 20% v/v ethanol) and permeate recovered from NF process were employed to study the effect of solvent type in the tannins extraction efficiency, at 3% w/v solids (dry basis) to solvent ratio and 30ºC. After the first extraction stage, lasting 300 min, the residue was treated again with the same volume of the same solvent for two sequential stages of 90 min each. The supernatant liquid of each stage was carefully removed, filtered and analyzed appropriately.

The performance of the extraction process, in the above cases, is expressed as the dimensionless recovery yield (Y %), i.e.,

$$Y = \frac{\text{Amount of compound recovered (mg/g dry weight)}}{\text{Maximum compound content in solid waste (mg/g dry weight)}} \text{x}100 \qquad (1)$$

2.4 Dead-end bench filtration experiments

A combined aqueous polyphenol solutions, obtained after three repeated extraction stages of 90 min each, from husk with distilled water (3 w/v in dry weight basis, 30ºC), were initially

clarified by micro-filtration, using sequentially 0.45μm and 0.2μm cellulose acetate flat sheet (Merck) dead end filtration. The bench-scale experimental set-up, described in detail elsewhere [36], was employed for the determination of pomegranate husk extract concentration by dead-end nanofiltration. The pressurized cylindrical Sepa-ST model membrane test cell (Osmonics Inc., Minnetonka, MN) can accommodate a membrane disc with effective area 12.7 cm^2, while the working volume is 300 mL. The test cell is equipped with a magnetic stirring device (Cimarec, Barnstead/Thermolyne) that is positioned above the membrane and held at small distance from the membrane without coming in contact with it. This stirrer rate was 250 rpm in all experiments for minimizing the phenomenon of concentration polarization. The working pressure into the cell is applied by a high-pressure nitrogen cylinder and controlled with a pressure regulator. The test cell was dipped into a water bath for maintaining a stable 30°C temperature.

A commercial polyamide-based NF membrane was used (NF270, Dow Filmtec) in this study. The membrane was initially compacted with deionized water at 15 bar for at least one hour and until stable flux was obtained, which was considered indicative of successful compaction. Water permeability measurements were performed prior to batch processing by NF at each transmembrane pressure (TMP) (4-14 bar) used for the concentration experiments. Experiments were performed under constant pressure of 4, 6, 8, 10, 12 and 14 bar; each batch was concentrated up to Volume Concentration Factor (VCF) of three. In cases of applied pressure 6, 8 and 10 bar, the same piece of NF membrane was used in ten sequential tests, without any membrane cleaning between each batch.

The VCF is expressed as the ratio of initial feed volume (V_0) over concentrate volume (V_C), where V_C = [(Feed Volume) – (Permeate Volume)] = [V_0 -V_P]; i.e.

VCF= (Feed Volume) / (Concentrate Volume) = $V_0/[V_0 - V_P]$ (2)

The pH effect was also examined under 8 bar and the same final VCF, by adjusting the pH of the initial extract with 10N NaOH to final values of 4, 5, 6, 7 and 8.

The total polyphenols ratio in each membrane stream was determined by measurements of their mass in the permeate ($m_{P,TPC}$), concentrate ($m_{R,TPC}$) and feed solution ($m_{F,TPC}$). The total polyphenols ratio (% TPR) in each stream is calculated accordingly as:

$$\%\,TPR = \left(\frac{m_{P\ or\ R,TPC}}{m_{f,TPC}}\right) x100 \qquad (3)$$

It is noted that all measurements and experiments reported in the following section were performed in triplicate. Average values are presented with the respective deviations (Table 1). Similarly, in plotted data the average values are provided with a bar designating deviation, which is within ± 6% in all cases.

3. Results and Discussion

3.1 Pomegranate waste characterization

The raw waste material characteristics are summarized in **Table 1.** The total weight of the waste materials (47.6±1.4 % w/w of initial wet fruit weight) is almost evenly distributed between husk (24.7±2 % w/w) and pith-carpellary membranes (22.9±0.8 % w/w). It was observed that dehydrating the samples by ordinary drying in an oven at 70ºC, or freeze drying, led to more than 90% w/w polyphenol losses (data not shown). The oxidation of pomegranate-husk polyphenols was studied by Al-Rawahi et al. [37]. They found that water could be more efficient in polyphenols recovery from the wet peels, whereas methanol from the dried ones; however, drying led to substantial polyphenols oxidation as well. The determination of polyphenols content in respect of fresh or wet weight has been also examined in other plant products [23]. The polyphenols content corresponding to dry weight was calculated after taking into consideration the wastes' moisture. The total polyphenols, hydrolysable tannins and flavonoids amounts are in accord with respective literature data [1]. As the pith-carpellary

membranes have smaller concentration of polyphenols (almost one-third in respect of husk), hereafter only the husk part was studied. The pith-carpellary membrane part could find other applications, such as feedstock and/or substrate for fermentations. Most of the polyphenols in husk are hydrolysable tannins (255.3±5.1 mg/gr dry weight), i.e. $Y_{HT/TPC}$= 91%, and only a small part (24.8±0.4 mg/gr dry weight) corresponds to total flavonoids (~ $Y_{TFC/TPC}$= 9%), as quantified with the analytical methods used herein. These results are in accord with similar literature data [1, 16], where water is used as solvent. The content of polyphenols in pomegranate husk may vary for several reasons, such as batch to batch variations, season and geographical location. The effect of such variations can be mitigated by expressing the results

Table 1. Analysis of pomegranate solid-waste used in this study and related parameters

Parameter	**Value** [b]
Pith-carpellary membranes (% w/w of fresh-fruit weight)	22.9±0.8
Husk (% w/w of fresh-fruit weight)	24.7±2.0
Total solid waste material (% w/w of fresh-fruit weight)	47.6±1.4
Moisture of pith-carpellary membranes (% w/w of fresh-fruit weight)	82.7±3.0
Moisture of husk (% w/w of fresh-fruit weight)	71.4±3.9
Total polyphenols in husk (mg/g dry weight)	280.3±5.0
Total polyphenols in pith-carpellary membranes (mg/g dry weight)	111.3±3.5
Total polyphenols in the mixed waste (mg/g dry weight)	195.8±2.6
Hydrolyzable tannins in husk (mg/g dry weight)	255.3±5.1
Total flavonoids in husk (mg/g dry weight)	24.8±0.4
Extract pH [a]	3.7
Extract Conductivity (μS/cm) [a]	313±15

a extracted with deionized water 3% w/v on dry basis, **b** average of three measurements and respective deviation.

in terms of dimensionless extraction efficiency (i.e. yield). Therefore, the maximum amount of polyphenols in the husk (280.3±5 mg/gr dry weight is considered hereafter as reference for determining the recovery yield.

3.2 Polyphenols extraction

3.2.1 Study of the extraction duration

Initially, the extraction of total polyphenols, hydrolysable tannins and flavonoids from 3% w/v in dry basis of pomegranate husk, with deionized water in a single stage, was examined. **Figure 1** shows that one and a half hour was sufficient to obtain a recovery yield of 60-65%. It is also evident that ~20-25% w/w of these compounds is recovered within few minutes time. This means that these are easily accessible by the water. The remaining polyphenols, hydrolysable tannins and flavonoids were extracted after an additional hour, indicating a slower diffusion from the inner part of the solids. As there is no significant difference in the time required for the recovery of flavonoids, hydrolysable tannins and polyphenols, and as the latter exist in larger amounts, hereafter only the recovery of the total polyphenols is examined.

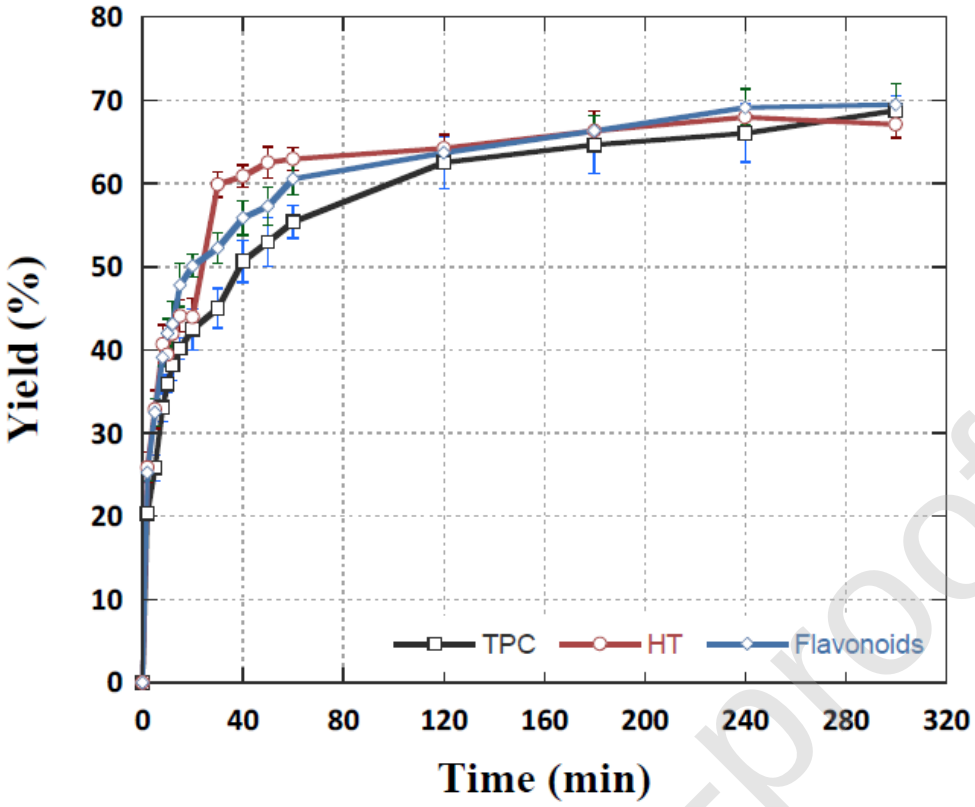


Figure 1. Yield of the pomegranate husk total polyphenols (TPC), hydrolysable tannins (HT) and flavonoids, employing water as extracting solvent, and solids to liquid ratio 3%w/v at 25°C.

3.2.2 Effect of solids to solvent ratio

The effect of solids to solvent ratio was examined in tests with samples at 2, 3, 5, 7, 9, 11 and 14 % w/v in dry basis equivalent. **Figure 2** suggests that the amount of polyphenols yield in a single extraction stage decreases from 70% to almost 40%, in an almost linear manner, with increasing solids to liquid ratio from 2 up to ~ 9% w/v. Interestingly, above the ratio 9% w/v the yield remains almost constant, which may be attributed to the maximum amount that can be dissolved in the available solvent. Additionally, it appears that the extraction time required to reach the plateau at higher solid to liquid ratios is reduced compared to that required at smaller ratios (data not shown). This trend may be due to the rapid solvent saturation by the readily available part of the polyphenols.

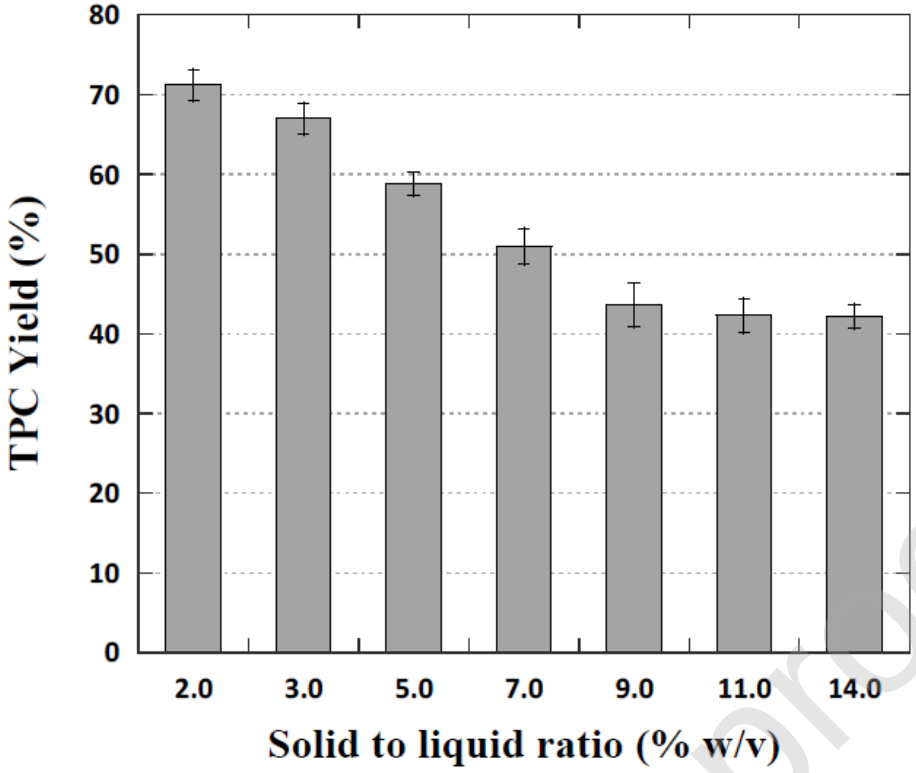


Figure 2. The effect of solids to liquid ratio on the yield of polyphenols from pomegranate husk with water as extraction solvent at 25ºC.

3.2.3 Effect of temperature

The effect of temperature on polyphenol yield was examined in a range of relatively low temperatures (15 to 55ºC), which is considered appropriate for this process. **Figure 3** shows that, with increasing temperature from 15 to 35ºC, there is a moderate increase in the TPC yield. Further temperature increase leads to a small reduction of TPC % yield. These results show that the increase in temperature can be beneficial up to a point for the polyphenols recovery, whereas further increase causes a decrease in the extraction yield [1, 8, 12]. This trend is in accord with previously reported results, regarding temperature effects on the extraction of polyphenols from pomegranate peels [1, 8, 12]. The negative effect of temperature increase could be attributed to the degradation of polyphenols, particularly above 35ºC [1, 12]. Apparently, with increasing temperature above ~30ºC, there is a greater increase of polyphenol

degradation rate compared to the modest increase of mass transfer rate that aids extraction [12]. Therefore, 30°C was chosen as an appropriate temperature for the polyphenol extraction as well as for the subsequent NF step.

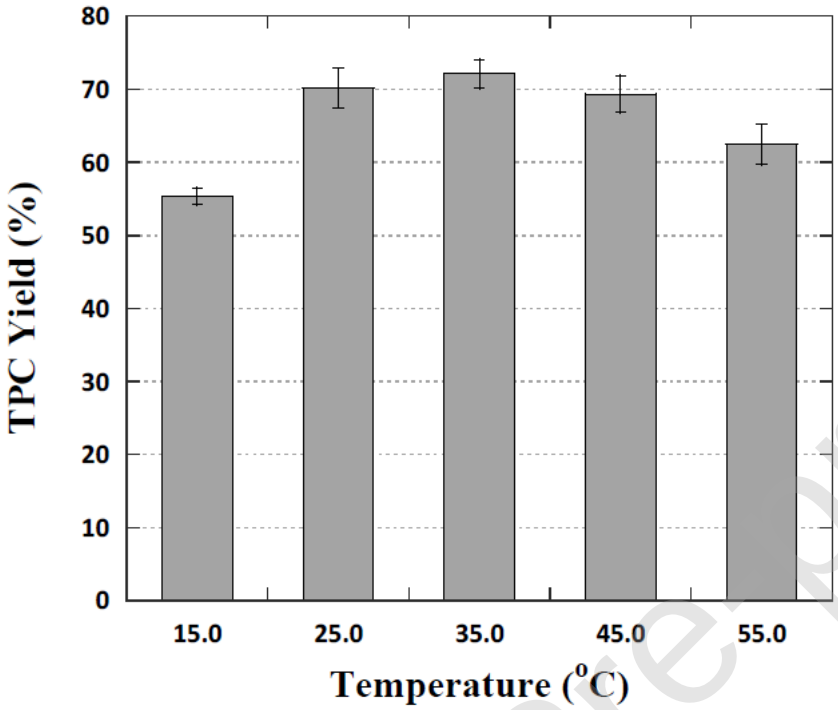


Figure 3. The effect of temperature on the yield of polyphenols from pomegranate husk with water as extraction solvent at solids to liquid ratio of 3% w/v.

3.2.4 Effect of solvent type and extraction stages

The effect of solvent type in polyphenols yield from pomegranate peels is examined in a number of studies [5, 14, 38]. The extraction yield of the total phenolics content depends on the type of solvent and composition in case of mixtures. Jingjing & Qipeng [14] found that an aqueous solution of 20% v/v ethanol was the most efficient for polyphenols recovery. Therefore, in this study deionized, tap water, and 10 and 20% aqueous ethanol solution were used; additionally, NF aqueous permeate was tested as possible solvent. **Figure 4** depicts the temporal variation of % TPC yield for the different solvents at 30°C, showing that (for all the examined solvents) extraction was completed within 60 to 120 min. The performance of

deionized water is satisfactory (compared to the best performing ethanol/water mixtures), rendering it a suitable candidate for practical applications. Deionised water is also preferable to tap water, as it leads faster to maximum yield (e.g. at approx. 60 min, compared to 180 min of tap water) and there are no undesirable interactions with ionic species present in tap water. The good performance of NF-permeate is particularly noteworthy; indeed, from the viewpoint of materials conservation it would be beneficial to recycle the NF-permeate.

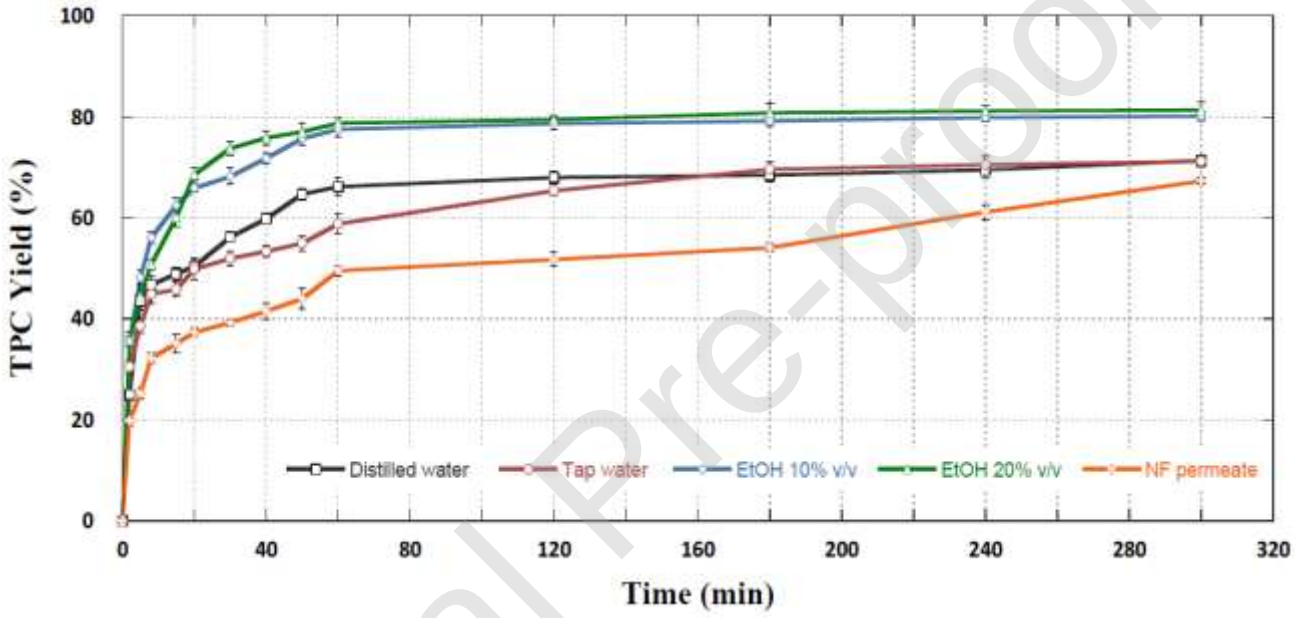


Figure 4. Temporal variation of % TPC yield for various solvents at 30ºC.

Figure 5 depicts the TPC yield from the husk for the various solvents for three repetitive stages; it is observed that two extraction stages are sufficient to extract more than 85% of the total polyphenols from the pomegranate husk. Additionally, the NF aqueous permeate recovered from the previous step can be efficiently utilized for the polyphenols recovery as it also leads to 85% TPC yield in just two steps.

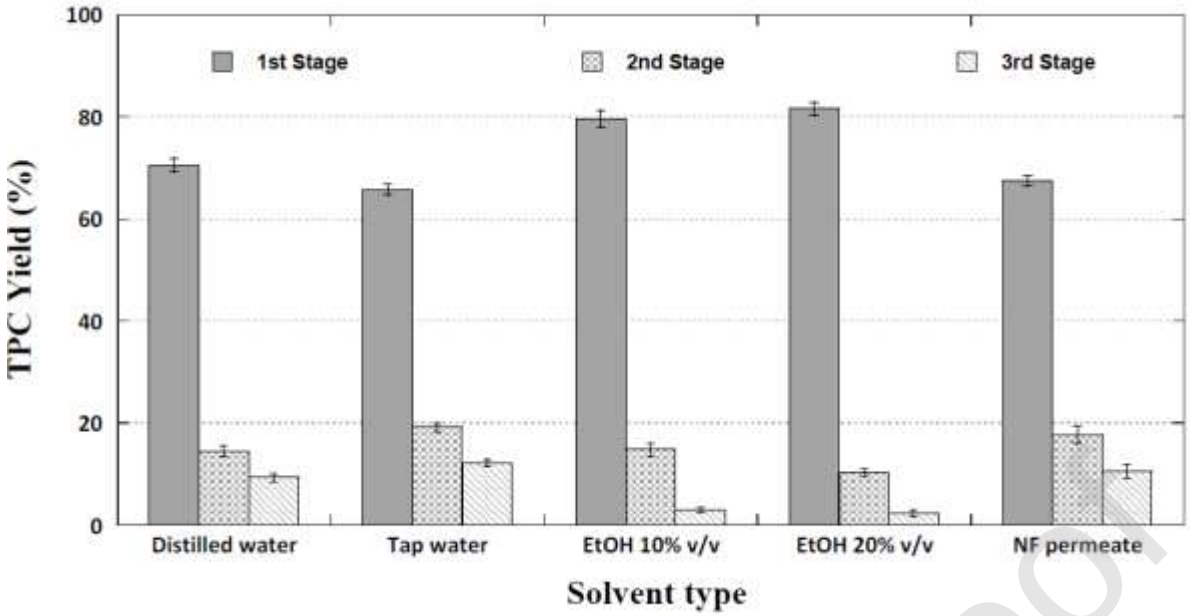
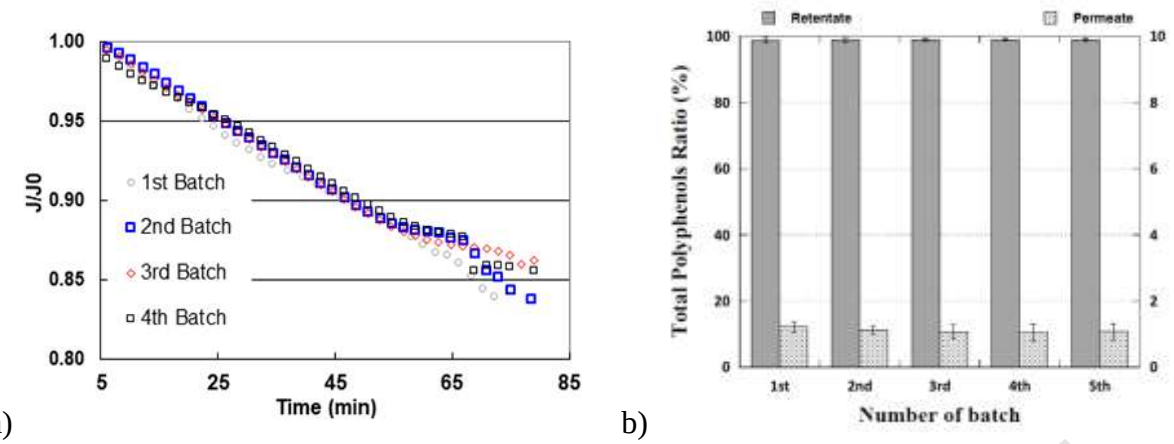


Figure 5. % TPC yield for three extraction stages using different solvents, at 30°C.

3.3 Polyphenols concentration by nanofiltration (NF)

The NF270 polyamide membrane used here is characterized by a nominal MWCO of 155 [39] -200 Da [31]. The hydraulic permeability L_p of new membrane specimens was initially determined in clean-water filtration experiments, prior to extracts filtration, by plotting the water flux measured at 30°C versus the applied TMP (data not shown). For the new membrane specimens employed in the present study, the clean-water permeability was determined to be 7.5 L/(m^2·h·bar), which is relatively close to other reported values; e.g. 8.5 [39] – 8.7 L/(m^2·h·bar) [40].

Figure 6a depicts the permeate-flux temporal variation for four sequential filtration batches under constant pressure (10 bar) with the same piece of membrane, whereas **Figure 6b** shows the total polyphenols ratio (%TPR) ~~retention~~ in both permeate and retentate under the same conditions. It is evident that the NF-membrane performance is excellent in respect of both polyphenols retention and filtration performance, suggesting that this membrane could be used over a prolonged period of time in a practical implementation of this process.



a) b)

Figure 6. NF membrane performance in the first five batches under 10 bar and 30ºC. **(a)** Temporal variation of normalized flux (J/J_0). **(b)** Total polyphenols ratio in both permeate and retentate streams (TPR %) in respect of feed (left axis: retentate; and right axis: permeate).

3.3.1 Effect of transmembrane pressure on polyphenols recovery

Figure 7 shows the total polyphenols ratio (%TPR) ~~concentration~~ in the NF permeate and retentate over the range of applied pressures 4 to 14bar. Throughout this range, total polyphenols are mainly retained in the retentate (> 96%), with an increasing trend, reaching a maximum (at ~10 bar) of ~99%.

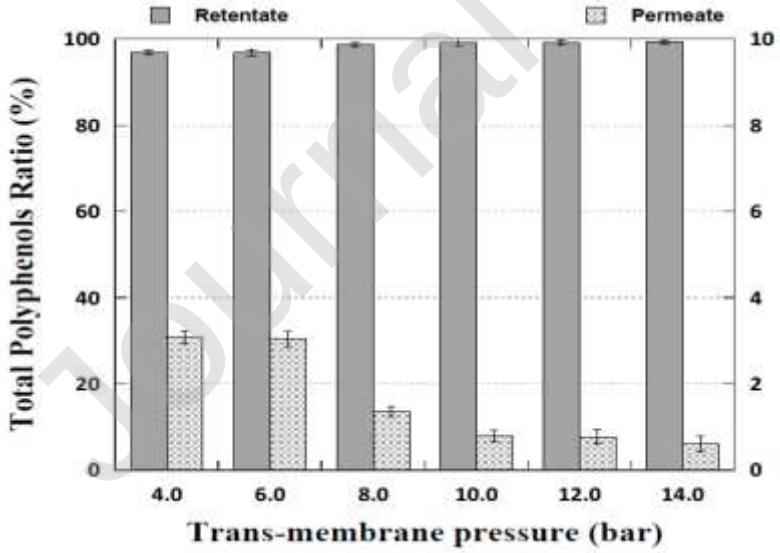


Figure 7. Total polyphenols ratio in the range of applied pressures 4 to 14bar (left axis: retentate; right axis: permeate).

As expected, the measured membrane permeability at the start of each applied pressure of the pomegranate-husk extract filtration tests (at 4.8 L/(m^2·h·bar)) was smaller than the hydraulic permeability of clean membrane (i.e. 7.5 L/(m^2·h·bar)). This reduction of permeability (and of the respective initial flux J_0) by 36% may be attributed mainly to concentration polarization phenomena [40]. Of particular interest is the reduction of dimensionless flux (J/J_0) with increasing solution concentration, shown in **Figure 8** for the range of applied pressures (i.e. 4 to14 bar). For a fixed applied pressure, this variation with increasing VCF is attributed primarily to concentration polarization effects [40 ~~35~~]. Additionally, for an increasing applied pressure from 4 to 10 bar, the level of dimensionless fluxes (at a fixed VCF) tends to be reduced somewhat, but it is not substantially different; however, a further pressure increase to 14 bar leads to a significant J/J_0 reduction. This trend may be attributed to greater membrane fouling, which is induced by the increased fluxes J in conjunction with concentration phenomena as discussed by Giacobbo et al [40]. From Figures 7 and 8 it is evident that applied pressures greater than 10 bar do not actually affect polyphenol retention, whereas fouling rate tends to increase.

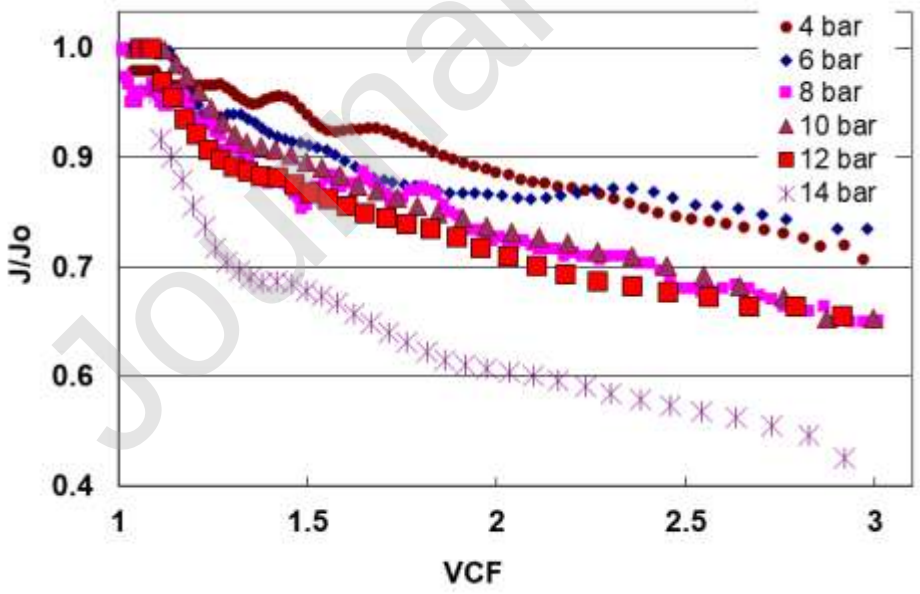


Figure 8. Variation of dimensionless flux, with respect to initial flux (J_0), versus VCF (during filtration) for various applied constant pressures (4-14bar) under the same initial solution pH 4.

3.3.2 Effect of pH on nanofiltration performance

Of particular interest is also the effect of pH on the normalized flux reduction, with increasing VCF (due to filtration), under the same constant pressure (10 bar), shown in **Figure 9**. It appears that acidic pH values, as those of the pomegranate-husk extract (Table 1), have a negative impact on the NF process efficiency, by reducing permeability. An explanation of this trend can be based [28, 31] on the effect of feed-solution pH to both the NF-membrane and the dissolved organic-compounds charge, which in turn affect the dissolved compounds transport through the membrane. For instance, charged compounds exhibit an increase of retention with increasing pH as a result of repulsion between the charged compound and membrane [31]. In case of polyamide thin-film composite membranes, it has been reported [41] that its charge characteristics influence the separation. The NF270 polyamide membrane has a pH operation range 3 to 9, and a ζ potential 2.4 and -21.6 at pH 3 and 7, respectively [31, 41], with an isoelectric point at pH~5 [30]. Hence, the NF270 membrane surface-charge is typically negative at pH values above 5, switching to positive values at lower pH [30, 42-43]. The polyphenols are negatively charged above pH 4, where the effect of their repulsion by the negatively charged membrane surface is enhanced. It should be noted that the total polyphenols retention was high for all the examined cases (TPR in the retentate ≥ 96%), even though membrane permeability was apparently affected at small pH. The reduced membrane permeability observed at small pH (< 5) may be attributed to its greater fouling propensity, associated with increased hydrophobic interactions involving the positively charged membrane surface and the non-ionized polyphenols. Furthermore, from the stand-point of process efficiency, it appears desirable to adjust the pH (~3.7) of the initial extract to a greater value,

before the NF concentration step, as this can lead to lower fouling rate, and reduced filtration time for a fixed VCF.

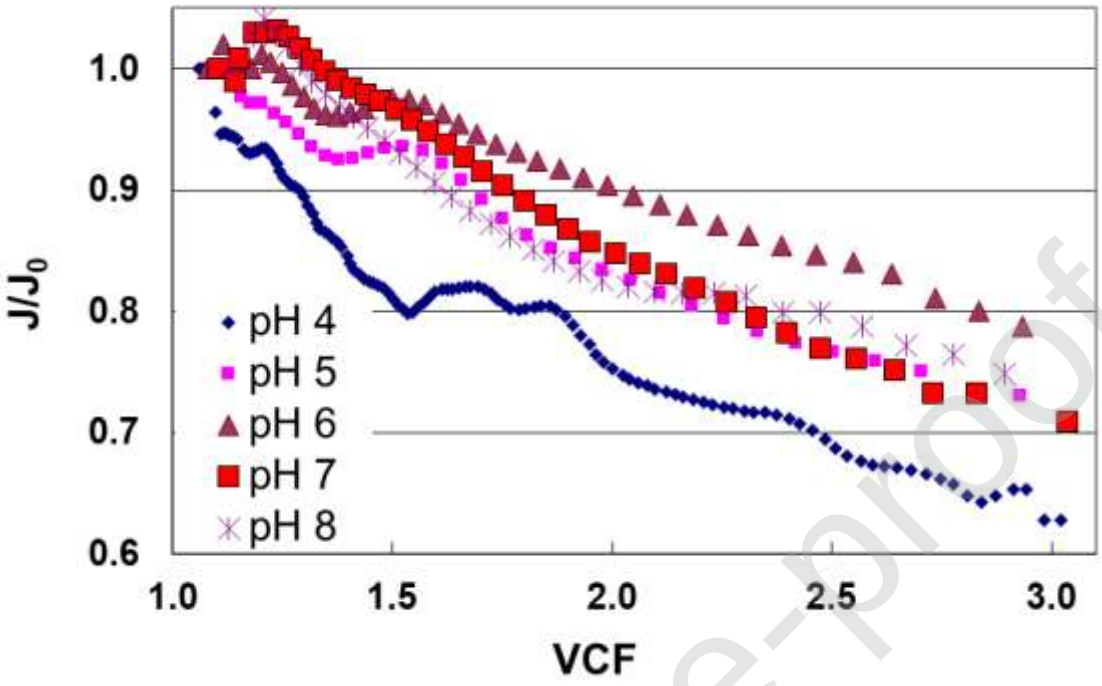


Figure 9. Variation of normalized flux, with respect to initial flux (J_0), versus VCF (during filtration), for various initial pH values, under the same constant pressure (10 bar).

4. Concluding remarks

This paper describes the results of a systematic bench-scale investigation aiming to develop an efficient process for valorization of solid wastes from the pomegranate-juice industry. The targeted product of this valorization process (i.e. polyphenols concentrate) is obtained through sequential aqueous extraction, from the relatively rich in polyphenols pomegranate husk, and concentration of the extract using a NF membrane process. The overarching objective in this particular process development is to identify a fairly narrow range of near optimum conditions, using criteria of *sustainability* (i.e. conservation of energy and solvent) and of *natural product/polyphenol integrity* (i.e. mild processing). The identified main process conditions (comprising two sequential extraction stages, using 3% w/v solids to water

ratio at 30°C, followed by appropriate NF-membrane concentration at 10 bar, with a possibility to recycle the NF permeate) appear to satisfy the above criteria. Moreover, the observed fairly stable performance of the employed NF membrane, in repeated separation tests without intermediate cleaning, is encouraging in respect of practical applications. An issue requiring additional attention is the possible need for pH adjustment of the feed to NF stage for improving membrane performance and process efficiency. Overall, the positive results of this laboratory study warrant further process development and optimization, toward a higher Technology Readiness Level (TRL > 5), involving pilot scale demonstration-testing of the integrated method in a realistic environment.

Credit author statement

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Declaration of Interests Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Çam, M. and Hişil Y. Pressurised water extraction of polyphenols from pomegranate peels, Food Chem. 123 (2010) 878-885.
2. Pareek, S., Valero D., and Serrano M. Postharvest biology and technology of pomegranate, J. Sci. Food Agricul. 95 (2015) 2360-2379.
3. Gullon, B., Pintado, M.E., Pérez-Álvarez, J.A., Viuda-Martos, M. Assessment of polyphenolic profile and antibacterial activity of pomegranate peel (*Punica granatum*) flour obtained from co-product of juice extraction, Food Control 59 **(**2016) 94-98.
4. Iqbal, S., Haleem, S., Akhtar, M., Zia-ul-Haq, M., Akbar, J. Efficiency of pomegranate peel extracts in stabilization of sunflower oil under accelerated conditions, Food Res. Internat. 41 (2008) 194-200.
5. Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., Cheng, S. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract, Food Chem. 96 (2006) 254-260.

6. Pathak, P.D., Mandavgane, S.A., and Kulkarni B.D. Valorization of Pomegranate Peels: A Biorefinery Approach, Waste Biom. Valor. 8 (2017) 1127-1137.
7. Singh, B., Singh, J.P., Kaur, A., Singh, N. Antimicrobial potential of pomegranate peel: a review, Intern. J. Food Sci. Technol. 54 (2019) 959-965.
8. Sood, A. and Gupta M. Extraction process optimization for bioactive compounds in pomegranate peel, Food Biosci 12 (2015) 100-106.
9. Saad, H., Charrier-El Bouhtoury, F., Pizzi, A., Rode, K., Charrier, B., Ayed, N. Characterization of pomegranate peels tannin extractives, Ind. Crops Prod. 40 (2012) 239-246.
10. Akhtar, S., Ismail, T., Fraternale, D., Sestili, P. Pomegranate peel and peel extracts: Chemistry and food features, Food Chem. 174 **(**2015) 417-425.
11. Kazemi, M., Karim, R., Mirhosseini, H., Abdul Hamid, A. Optimization of pulsed ultrasound-assisted technique for extraction of phenolics from pomegranate peel of Malas variety: Punicalagin and hydroxybenzoic acids, Food Chem. 206 **(**2016) 156-166.
12. Machado, A.P.D.F., Sumere, B.R., Mekaru, C., Martinez, J., Bezerra, R.M.N., Rostagno, M.A. Extraction of polyphenols and antioxidants from pomegranate peel using ultrasound: influence of temperature, frequency and operation mode, Intern. J. Food Sci. Technol. 54 (2019) 2792-2801.
13. Aqil, F., Munagala, R., Vadhanam, M.V., Kausar, H. Jeyabalan, J., Schultz, D.J., Gupta, R.C. Anti-proliferative activity and protection against oxidative DNA damage by punicalagin isolated from pomegranate husk, Food Research Intern. 49 (2012) 345-353.
14. Jingjing, L. and Qipeng Y. A new method for ellagic acid production from pomegranate husk, J. Food Process Engin. 31 (2008) 443-454.
15. Qu, W., Pan Z., and Ma H. Extraction modeling and activities of antioxidants from pomegranate marc, J. Food Engin. 99 (2010) 16-23.
16. Çam, M. and İçyer N.C. Phenolics of pomegranate peels: extraction optimization by central composite design and alpha glucosidase inhibition potentials, J Food Sci. Technol. 52 (2015) 1489-1497.
17. Amyrgialaki, E., Makris, D.P., Mauromoustakos, A., Kefalas, P. Optimisation of the extraction of pomegranate (*Punica granatum*) husk phenolics using water/ethanol solvent systems and response surface methodology, Ind. Crops Prod. 59 **(**2014) 216-222.
18. Nawaz, H., Shi, J., Mittal, G.S., Kakuda, Y. Extraction of polyphenols from grape seeds and concentration by ultrafiltration, Sep. Purif. Technol. 48 (2006) 176-181.
19. Kontogiannopoulos, K.N., Patsios, S.I., Mitrouli, S.T., Karabelas, A.J. Tartaric acid and polyphenols recovery from winery waste lees using membrane separation processes, J. Chem. Technol. Biotechnol.. 92 (2017) 2934-2943.
20. Kalbasi, A. and Cisneros-Zevallos L. Fractionation of monomeric and polymeric anthocyanins from concord grape *(Vitis labrusca L.)* juice by membrane ultrafiltration, J. Agricul. Food Chem. 55 (2007) 7036-7042.
21. Santamaría, B., Salazar, G., Beltrán, S., Cabezas, J.L. Membrane sequences for fractionation of polyphenolic extracts from defatted milled grape seeds, Desal. 148 (2002) 103-109.
22. Cheung, L.M. and Cheung P.C.K.,Mushroom extracts with antioxidant activity against lipid peroxidation, Food Chem. 89 (2005) 403-409.

23. Gu, H.-F., Li, C.-M., Xu, Y.-j., Hu W.-f, Chen, M.-h., Wan, Q.-h. Structural features and antioxidant activity of tannin from persimmon pulp, Food Res. Intern. 41 (2008) 208-217.
24. Prodanov, M., Garrido, I., Vacas, V., Lebrón-Aguilar, R., Dueñas, M., Gómez-Cordovés, C., Bartolomé, B.Ultrafiltration as alternative purification procedure for the characterization of low and high molecular-mass phenolics from almond skins, Anal. Chim. Acta 609 (2008) 241-251.
25. Yu, Z.-R., Hung, C.-C., Weng, Y.-M., Su, C.-L., Wang, B.-J. Physiochemical, antioxidant and whitening properties of extract from root cortices of mulberry as affected by membrane process, LWT - Food Sci. Technol. 40 (2007) 900-907.
26. Todisco, S., P. Tallarico, and Gupta B.B. Mass transfer and polyphenols retention in the clarification of black tea with ceramic membranes, In. Food Sci. Emerg. Technol. 3 (2002) 255-262.
27. Li, P., Wang, Y., Ma, R., Zhang, X. Separation of tea polyphenol from Green Tea Leaves by a combined CATUFM-adsorption resin process, J. Food Engin. 67 (2005) 253-260.
28. Dammak, I., Neves, M. A., Nabetani, H., Isoda, H., Sayadi, S., Nakajima, M. Effect of pH condition on the retention of oleuropein in aqueous solution by nanofiltration membrane, Sep. Sci. Tech. 49 (2014) 2289-2302.
29. Qiao, X., Zhang, Z., and Ping, Z., Hydrophilic modification of ultrafiltration membranes and their application in *Salvia Miltiorrhiza* decoction, Sep. Purif. Technol. 56 (2007) 265-269.
30. Childress, A. E. and Elimelech, M. Effect of solution chemistry on the surface charge of polymeric reverse osmosis and nanofiltration membranes, J. Membr. Sci. 119 (1996) 253-268.
31. Braeken, L., Bettens, B., Boussu, K., Van der Meeren, P., Cocquyt, J., Vermant, J., Van der Bruggen, B. Transport mechanisms of dissolved organic compounds in aqueous solution during nanofiltration. J. Membr. Sci. 279 (2006) 311-319.
32. Luo, J. and Wan, Y. Effects of pH and salt on nanofiltration-a critical review. J. Membr. Sci. 438 (2013) 18-28.
33. Box, J.D., Investigation of the Folin-Ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters, Water Res. 17 (1983) 511-525.
34. Zhishen, J., Mengcheng, T., Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chem. 64 (1999) 555-559.
35. Willis, R.B., Allen, R. Improved method for measuring hydrolyzable tannins using potassium iodate, Analyst 123 (1998) 435-439.
36. Sioutopoulos D.C., Karabelas A.J. Correlation of organic fouling resistances in RO and UF membrane filtration under constant flux and constant pressure. J. Membr. Sci. 407-408 (2012) 34–46.
37. Al-Rawahi, A.S., Rahman, M.S., Guizani, N., Essa, M.M., Chemical Composition, Water Sorption Isotherm, and Phenolic Contents in Fresh and Dried Pomegranate Peels, Drying Technol. 31 (2013) 257-263.
38. Wang, Z., Pan, Z., Ma, H., Atungulu G.G. Extract of phenolics from pomegranate peels, Open Food Sci. J.. 5 (2011)17-25.
39. Boussu, K., Zhang, Y., Cocquyt, J., Van der Meeren, P., Volodin, A., Van Haesendonck, C., Martens, J. A.,Van der Bruggen, B. Characterization of polymeric nanofiltration

membranes for systematic analysis of membrane performance, J. Membr. Sci. 278 (2006), 418-427.

40. Giacobbo A., Bernardes A.M., Rosa M.J.F., de Pinho M.N. Concentration Polarization in Ultrafiltration/Nanofiltration for the Recovery of Polyphenols from Winery Wastewaters, Membr. 8 (2018) 46.
41. Dalwani, M., Benes, N.E., Bargeman, G., Stamatialis, D., Wessling, M. Effect of pH on the performance of polyamide/polyacrylonitrile based thin film composite membranes, J. Membr. Sci. 372 (2011), 228-238.
42. Bandini, S. Modelling the mechanism of charge formation in NF membranes: Theory and application, J. Membr. Sci. 264 (2005), 75-86.
43. Bandini, S. and Mazzoni, C. Modelling the amphoteric behaviour of polyamide nanofiltration membranes, Desal.184 (2005) 327–336.