Original article

Clarification of pomegranate juice by ultrafiltration: study of juice quality and of the fouling mechanism

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Clarification of pomegranate juice by ultrafiltration: study of juice quality and of the fouling mechanism.

Abstract — **Introduction**. Ultrafiltration (UF) is a single-unit operation for the clarification and fining of fruit juices. The purpose of the UF is to remove suspended solids as well as haze-inducing and turbidity-causing substances to obtain a clear juice during storage. Specifically, the polymerization of phenolic compounds and their interaction with other components (*e.g.,* proteins) could cause a haze complex and turbidity in fruit juices, which can foul the ultrafiltration membrane. **Materials and methods**. Fresh pomegranate juice was clarified by the ultrafiltration process on a laboratory scale. In experimental tests performed according to the total recycle and the batch concentration mode, the effects of transmembrane pressure (TMP) and enzyme pre-treatment on permeation flux and quality of juice were studied. **Results**. With the total recycle mode, the effect of TMP on the color and clarity of clarified pomegranate juice was significant. The initial color of the raw pomegranate juice was reduced from 74% to 33% and the clarity decreased from 77% to 42% by UF when the TMP increased from (1 to 3.6) bar. Total phenolic rejection decreased from 45% to 21% when the TMP rose from (1 to 2) bar and remained constant above this value. With the batch concentration mode at TMP = 2 bar and velocity 1 $m \cdot s^{-1}$, the enzymatic treatment (5 $U \cdot mL^{-1}$, 300 min, T = 20 °C) of pomegranate juice provided the highest permeate flux, a decrease in total phenolics of 50% and an increased clarity of 30%. Fouling of the UF membrane during pomegranate juice processing is mainly due to the retention of polyphenols and/or proteins; thus, several blocking mechanisms were studied, using a recently developed membrane-fouling model. Analysis revealed that the membrane separation process was controlled by the gel layer mechanism of raw pomegranate juice and complete pore blocking mechanism with enzymatic pre-treatment.

Tunisia / *Punica granatum* / fruit juices / clarifying / ultrafiltration / phenolic compounds / membranes / fouling / processing / laccase

Clarification du jus de grenade par ultrafiltration : étude de la qualité du jus et du mécanisme de colmatage.

Résumé — **Introduction**.L'ultrafiltration (UF) est un procédé simple pour la clarification et pour l'affinage de jus de fruits. Son objectif est d'éliminer les matières en suspension ainsi que les substances induisant trouble et turbidité pendant son stockage. Plus précisément, la polymérisation des composés phénoliques et leur interaction avec d'autres composants (des protéines, par exemple) pourraient causer du trouble et de la turbidité dans les jus de fruits, conduisant au colmatage de la membrane d'ultrafiltration. **Matériel et méthodes**. Un jus de grenade frais a été clarifié par ultrafiltration. Les essais expérimentaux ont été effectués en mode de recyclage total ou en batch par renvoi du retentât dans la cuve d'alimentation. Les effets de la pression transmembranaire (PTM) et d'un prétraitement enzymatique sur le flux de perméation et la qualité des jus ont été étudiés. **Résultats**. Avec le mode de recyclage total, l'effet de la PTM sur la couleur et la clarté du jus de grenade clarifié ont été significatifs. La couleur du jus de grenade brut a été réduite de 74 % à 33 % et sa clarté a diminué de 77 % à 42 % lorsque la PTM est passée de (1 à 3,6) bar. Le rejet des composés phénoliques a diminué de 4 5 % à 21 % lorsque la PTM est passée de (1 à 2) bar et a été maintenu constant au-dessus de 2 bar. En mode batch et pour une PTM de 2 bar et une vitesse de 1 $m \cdot s^{-1}$, le traitement enzymatique (5 $U \cdot mL^{-1}$, 300 min, T = 20 °C) du jus de grenade a amélioré le f lux du perméat et a entrainé une diminution des composés phénoliques totaux de 50 % et une augmentation de la clarté de 30 %. Le colmatage de la membrane d'UF lors du traitement de jus de grenade est principalement dû à la rétention des polyphénols et/ou de protéines ; par conséquent, plusieurs mécanismes de colmatage ont été étudiés, en utilisant un modèle de colmatage de membrane récemment développé. L'analyse a révélé que le procédé de séparation membranaire a été contrôlé par le mécanisme de la couche de gel pour le jus de grenade brut et par un mécanisme de blocage complet des pores pour le jus avec prétraitement enzymatique.

Tunisie / *Punica granatum* / jus de fruits / clarification / ultrafiltration / composé phénolique / membrane / encrassement / traitement / laccase

1. Introduction

Pomegranate *(Punica granatum* L.) is one of the oldest edible fruits, widely grown in many countries (Iran, India, Turkey, Tunisia, Pakistan and Spain). In Tunisia, it is cultivated in many regions such as Tunis, Zaghouan, Djerba, Gabes and Sfax. Its beneficial effects are attributed to the antioxidative properties of pomegranate phenolic compounds as well as to those of sugar-containing polyphenolic tannins [molecular weight (MW) = 500–3,000 Da] and anthocyanins (MW = 207.247 Da) There is growing interest in this fruit not only because of its pleasant taste but also because of scientific evidence that suggests the fruit's health-beneficial effects due to its composition. The recent studies on health benefits of consumption of pomegranate juice involve prevention or treatment of atherosclerosis, reduction of blood pressure, inhibition of cancer cell proliferation, anti-inflammatory effects, stimulation of T-cell functions and treatment of diabetes [1–3]. On the basis of these characteristics, pomegranate processing has recently been gaining more importance. Consumer acceptance of pomegranate juice depends on the combination of several quality attributes that are related to the physicochemical properties including color, acidity and flavor. Pomegranate fruit is consumed directly as fresh fruit or as juice. However, processing a safe, stable and high-quality product which has the characteristics of fresh fruit, aroma and nutritional value is complicated; also, production has not been industrialized yet.

The edible part of the fruit contains considerable amounts of phenolic compounds. The bound phenolic constituents give color, astringency and bitterness to the pomegranate juice [4]. Since the main problem with pomegranate juice is stability, there is a need for research to solve this problem and, in addition, the preservation of color of the pomegranate juice [5]. According to the literature, the current methods for polyphenol elimination involve liquid extraction with organic solvents. However, these methods require high temperature to increase the extraction rate and yield but may denature the polyphenols [6].

Recent efforts have been devoted to evaluating processes based on membrane filtration due to their potential advantages in comparison with traditional processes, such as avoiding the use of fining agents (clarification) and not using high temperatures (concentration). The introduction of these technologies into the processing cycle of fruit juice can produce a clarified juice of high quality and fresh taste, nature-identical and additive-free. In particular, ultrafiltration (UF) represents a valid alternative to offer the possibility of clear filtering, sterile juices in one step, with and without the addition of clarifiers. Other advantages of the UF-based process are easy automation, lower labor and energy costs, and mild operating conditions. However, membrane filtration has the disadvantage of fouling, resulting in decline in performance. Indeed, a major limiting factor in UF is the flux decline with time due to the effects of membrane fouling, that reduce process efficiency [7]. The membrane structure and the molecular weight cut-off have an important role in the solute-membrane physicochemical interaction. All the compounds whose size is greater than the membrane pore size are accumulated on the membrane surface by convective transport, forming a concentration gradient known as the "polarized layer". The solute concentration increases on the interface until a dense cross-linked layer of deposited particles is formed round the membrane surface, which is known as the "gel layer". This dynamic layer is modified by operating conditions, such as cross-flow velocity, transmembrane pressure (TMP) or concentration of feed solution [6, 8, 9]. In juice UF, membranes retain large species such as micro-organisms, lipids and colloids, while small solutes such as, for example, vitamins, salts, sugars and free phenolic compounds flow through the membrane together with water. Membrane fouling is caused mainly by large constituents of the juice such as pectin and polyphenol-protein complexes, which subsequently clog up the membranes. Treatment with enzymes helps to guarantee the complete hydrolysis of pectic substances, to minimize the risk of fouling of the costly membranes [10–15] and removal/degradation of polyphenolic compounds that are responsible for haze and sediment formation as well as browning during storage of juice [5, 16–18]. There are several studies in which enzyme treatment has been proven to show an improvement in juice clarification and stability over the conventional process. For example, laccase treatment was important in the removal of polyphenols [5]. During the enzymatic treatment, laccase catalyzes the oxidation of juice phenols to o-quinines, which are highly reactive compounds. They undergo spontaneous polymerization to produce high-molecular-weight compounds (brown pigments) [19]. These compounds could be discarded by centrifugation. On the other hand, low-molecular-weight phenols (oligomers) remain in the supernatant and could lead to a darkening of the juice color which could easily be removed by membrane filtration [5, 20].

The objective of our investigation was to study the effect of transmembrane pressure and enzyme pre-treatment on the permeate flux, membrane fouling mechanism and juice quality on a laboratory scale. The quality parameters of clarified juice were clarity, color and phenolic content.

To determine the impact of transmembrane pressure and enzyme pre-treatment, several blocking mechanisms of a UF membrane using a recently developed membrane-fouling model [21, 22] were investigated.

Different fouling mechanisms were visualized (*figure 1*) [23]. When the pore size is smaller than the size of suspended particles in juice, the particle deposit on the external membrane surface entrances results in a complete or partial pore blocking (*figures 1a, 1b*), reducing membrane porosity and permeability. Subsequently, the accumulated particles can form aggregates. These particles grow in time, leading to the formation of a secondary membrane or cake (*figure 1c*). The thickness of this secondary membrane is responsible for an additional mass transport resistance which can be effectively controlled by an appropriate choice of fluid-dynamic conditions. The particles with a size range much smaller than that of the membrane pores will pass through. These particles may be adsorbed and deposited (*figure 1d*), leading to the formation of a colloidal film on the internal pore surface [24]. The pore radius is reduced and the hydraulic resistance is increased.

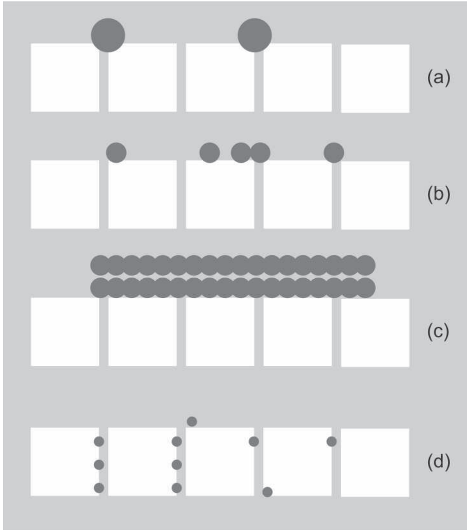


Figure 1
Mechanism for membrane fouling used for ultrafiltration treatment of pomegranate juice: (a) complete pore blocking; (b) partial pore blocking; (c) cake filtration; (d) internal pore blocking [23].

A mathematical model which is able to describe the flux decline (dJ/dt) based on an equation applied to dead-end filtration mechanisms at constant pressure filtration has been proposed by Hermia [21] and modified by Field *et al.* [22]. This model accounts for the fouling mechanism involved in cross-flow filtration, and has been used to describe fouling mechanisms in orange and pineapple juice [7, 25]. The general differential equation is the following: $-\frac{dJ}{dt} = (k(J - J_{\text{lim}}))/(J^{n-2})$, where J_{lim} represents the limit flux obtained in steady-state conditions; k and n are phenomenological coefficients depending on the fouling mechanism. Values for the parameter n, depending on the type of fouling, are the following (*figure 1*): complete pore blocking (n = 2), partial pore blocking (n = 1), internal pore blocking (n = 3/2) and gel layer formation (n = 0). The constant k depends on the transmembrane pressure, the dynamic permeate viscosity and the blocked areas per unit of permeate flux and the membrane resistance, R_m [26]. The parameters considered by these models have a physical meaning and contribute to the comprehension of the mechanisms involved in membrane fouling.

2. Materials and methods

2.1. Fruit origin

Pomegranate fruits (*Punica granatum* L.) of proper maturity and ripeness were supplied from a local market in Tunisia.

2.2. Juice extraction

The pomegranate fruits were washed and then manually cut up. Following peeling, the outer leathery skin that encloses the fleshy seeds was removed. The remaining pomegranate seeds were centrifuged by a Philips Electric juice centrifuge to obtain fresh juice. The pH and total soluble solids of the juice obtained were 4.1 and 15 °Brix, respectively. Raw juices were kept frozen at –20 °C until analysis [27].

2.3. Juice analysis

Protein concentration in the pomegranate juice was measured by the Kjeldahl method using the conversion factor of 6.25. Pectin was determined by a method described by Englyst *et al.* [28]. Atomic absorption spectrophotometry (for Cu, Fe, Zn, Mg and Ca) and flame spectrophotometry (Sherwood, model 410) (for Na and K) were used to measure mineral salts. Lipids were determined using the Soxhlet system [29]. The total sugar was measured by the colorimetric method using dinitrosalicilic acids (DNS). Total phenolics were determined with Folin-Ciocalteau (FC) reagent, and referred to as $mg \cdot L^{-1}$ of gallic acid [30]. Color (as absorbance at 420 nm on samples diluted by a factor of 5) [5, 16] and clarity (as absorbance at 650 nm) [5] values were measured using a Shimadzu UV-VIS scanning spectrophotometer (UV-2101-PC) [27].

2.4. Enzymatic pre-treatment

Before ultrafiltration (UF), the juice was treated at 20 °C with *Fomes fomentarius* laccase (MUCL 35117) at a concentration of $5\ U \cdot mL^{-1}$ for 300 min. The optimum conditions of this pre-treatment were determined in a previous work using response surface statistical methodology [27]. At 20 °C, the viscosity values for the raw and treated juice are, respectively, (3.49 and 2.09) $mPa \cdot s^{-1}$.

2.5. UF unit and procedures

Pomegranate juice was clarified by using a laboratory pilot unit equipped with a cross-flow filtration system implementing a tubular mineral CARBOSEP M2 membrane (stainless 31b membrane module, Tech-Step, France) (*table I*). The equipment consists of a feed tank, a thermometer, two manometers for the measurement of inlet and outlet pressures and a flow meter of 0–2 $L \cdot min^{-1}$ for measurement of the axial feed flow rate (Q_f) and, consequently, the tangential fluid velocity (0–3.8 $m \cdot s^{-1}$). All experiments were conducted at 20 °C. Transmembrane pressure (TMP) was controlled by the valve on the retentate side to give a pressure (P) of 1–4 bar and calculated as $TMP = (P_{in} + P_{out})/2$. Ultrafiltration experiments were performed according to the total recycle and the batch concentration mode. In the former, the experimental trials were devoted to the investigation of the effect of the transmembrane pressure on the flux performance of the system and quality of juice. In this case, the permeate was continuously recycled to the feed tank to ensure a steady state in the volume and composition of the feed. In the batch concentration mode, the UF system was operated at a TMP of 2 bar, at a tangential fluid velocity of 1 $m \cdot s^{-1}$ to study the effect of enzymatic treatment on the fouling during the clarification process up to a volume reduction factor (VRF) of about 6 units; VRF is defined as the ratio between the initial feed volume and the volume of the resulting retentate.

At the end of each run, cleaning-in-place was used for the membrane module according to the manufacturer's recommendations using caustic soda (5–10 $g \cdot L^{-1}$; T = 80–85 °C; operating time = 30 min; TMP = 2–3 bar) and nitric acid (3– 5 $mL \cdot L^{-1}$; T = 55–60 °C; operating time = 30 min; TMP = 2–3 bar). The membrane was exposed to this washing sequence until the original water flux was restored.

Table I.
Characteristics of the CARBOSEP ultrafiltration membrane used for clarification of raw pomegranate juice.

Nominal molecular weight cut-off (kDa)	Membrane area (cm^2)	Length (cm)	Inner diameter (mm)	pH operating range	Water permeability ($L \cdot hm^{-2} \cdot bar^{-1}$)
15	75	40	6	1–14	60

Table II.
Physicochemical analysis of unclarified pomegranate juice (amount·100 g^{-1} of raw juice).

Proteins	Lipids	Total sugar	Pectin	Total phenols	Mineral salts	Cu	Fe	Zn	Mg	Na	Ca	K
	(g)			(g)					(mg)			
0.96–1.20	0.30–0.40	10.18–12.02	traces	1.2–1.8	0.3–0.5	≤ 0.08	2.0–3.2	0.3–0.4	3–4.4	25–34	16–17	356–466

The rejection (R%) of the membrane is estimated by the relation: $R = 100\left(1 - \frac{C_p}{C_0}\right)$, where C_p is the end concentration in the permeate ($mg \cdot L^{-1}$) and C_0 is the initial concentration of the feed ($mg \cdot L^{-1}$).

2.6. Procedure for estimation of parameters n and k

The general differential equation:

$$\frac{dJ}{dt} = k(J - J_{\lim})/J^{n-2} \quad (1)$$

is solved by an explicit, improved Euler scheme. The general form of this differential equation is $\frac{dJ}{dt} = f(J, t)$. The numerical scheme used starts with an Euler step, giving a provisional value for J_{i+1} at the next time t_{i+1}:

$$J_{i+1} = J_i + hf(t_i, J_i) \quad (2)$$

The taken step actually looks like an Euler step, but with f replaced by the average of f at the starting point of the step and f at the provisional point:

$$J_{i+1} = J_i + \frac{h}{2}[f(t_i, J_i) + f(t_{i+1}, J_{i+1})] \quad (3)$$

The differential equation (1) displays two parameters, k and n, that have to be determined. The basic operation of the optimization process is the minimization of the functional built from both experimental results and numerical simulation in order to characterize the "distance" between them. This minimized functional ℓ depends on the vector of the parameters (n, k) and can be expressed as the sum of the elementary functional ℓ_j, defined for each experimental test *(j)* based on:

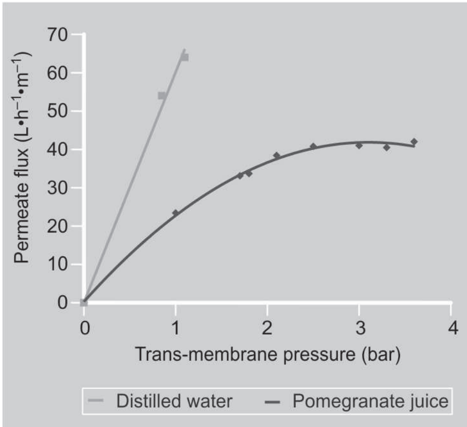
$$\ell(n, k) = \sum_j \ell_j(n, k) \quad (4)$$

The optimization procedure is as follows. The parameter n is first assigned to some particular values (n = 0, 1, 1.5 and 2). Then, k is adjusted to minimize the deviation between experimental and simulated data.

3. Results and discussion

Physicochemical analysis of unclarified pomegranate juice shows that there are three compounds, protein, lipids and phenolic compounds, responsible for fouling layer formation (*table II*). Proteins and polyphenolic compounds can combine to form soluble complexes, which may have colloidal sizes, and may scatter light, and can result in sediment formation [31].

Figure 2
Effect of the transmembrane pressure (TMP) used for ultrafiltration treatment of pomegranate juice on the permeate flux (temperature, T = 20 °C; tangential velocity, $V_m = 1\ m\cdot s^{-1}$).



3.1. Effect of transmembrane pressure on the permeate flux and clarified raw juice quality

Ultrafiltration experiments, carried out according to the total recycle mode, were performed in order to study the effect of transmembrane pressure (TMP) on the permeate fluxes and permeate properties. Permeate flux values *versus* the applied TMP (*figure 2*) show that, for small pressures, the solvent flux is proportional to the applied pressure. As the pressure is increased, the flux shows a deviation from linear flux-pressure behavior and becomes independent of pressure. Under these conditions a limiting flux is reached at a TMP value of about 2 bar, and any further pressure increase determines no significant increase in the permeate flux. The existence of a limiting flux can be related to the fouling.

The effect of TMP on color and clarity of clarified pomegranate juice was significant (*table III*). Color is an important sensory attribute. The initial color of the raw pomegranate juice was reduced from 74% to 33% by UF when the TMP increased from (1 to 3.6) bar. Under the effect of pressure, the pigments responsible for the color can pass through the membrane and therefore they cause an increase in color [16].

Clarity is another important sensorial quality parameter for clarified juices. It may be observed that the initial clarity value of the raw pomegranate juice was improved by UF. The clarity decreased from 77% to 42% by UF when the TMP increased from (1 to 3.6) bar. This decrease in the clarity can be related to the increase in color when the transmembrane pressure increased.

Total phenolic rejection decreased from 45% to 21% when the TMP rose from (1 to 2) bar (*table III*), mainly because the more TMP, the higher the amount of solute flowing through the membrane. Increasing the TMP from (2 to 3) bar resulted in an increase in the amount of deposited fouling materials on the membrane surface, and consequently increasing total phenolic rejection. The fouling formed at a higher TMP can prevent the solute from crossing the membrane and, therefore, reduce the amount of phenolic compounds in the permeate, resulting in a decrease in the astringency and an increase in stability of the pomegranate juice. Mirsaeedghazi *et al.* showed that clarification of pomegranate juice using membranes decreased phenolic content for all

Table III.
Effect of transmembrane pressure (TMP) on the color clarity and phenolic compound rejection of clarified pomegranate raw juice at tangential velocity (V_m) = 1 $m\cdot s^{-1}$.

Transmembrane pressure (bar)	Total phenolic rejection	Color reduction	Clarity improvement
		(%)	
1.00	45.45	74.45	76.92
1.70	25.72	46.50	56.41
2.05	21.23	42.13	55.12
2.45	23.31	38.00	51.92
3.60	26.51	32.75	41.66

membranes used [32]. The rejection factors vary between 25% and 50%.

3.2. Enzyme influence on the permeate flux and the quality of clarified juice

When studying the performance batch system while processing the pomegranate juice with and without laccase pre-treatment, permeate flux *versus* time obtained with raw juice and juice pre-treated with enzyme decreased rather slowly at the initial stage and achieved a quasi-steady state afterwards (*figure 3*). The slow decline of permeate flux, attributed to fouling, could be explained by the fact that, in the early stage of UF, permeate flux is controlled by the internal fouling [11]. Membrane fouling is caused mainly by large constituents of the juice such as proteins and polyphenol-protein complexes. The pre-treatment improved the permeation flux (*figure 3*).The steady-state permeate flow increased by around 40% with enzyme treatment. Similar results were achieved with umbu juice pre-treated with enzyme before UF [33]. The final permeate of umbu juice increased by 25–35% with enzyme treatment at low velocity and high pressure or at high velocity and high pressure. Typical behavior was observed with apple juice pre-treated with enzyme followed by pasteurization. The permeate flux was increased by about 81% under the applied TMP of 2 bar and the cross-flow velocity of 2.5 $m \cdot s^{-1}$ [11].

The characteristics of the pomegranate juices obtained by different treatments show that the total phenolic rejection capacity was enhanced by enzymatic treatments (*table IV*). Total phenol rejection was 21% for ultrafiltered, 38% for laccase-treated and 49% for laccase-UF-treated juice. It was reported by Giovanelli and Ravasini that the combined laccase-UF process removed 70% (cut-off value 15 kDa) of the total phenolics in apple juice [34].

The application of UF caused a notable decolorization in laccase-treated samples. However, laccase-UF-treated and only UF-treated pomegranate juice showed the same decrease in their color (35%) compared with untreated samples (*table IV*). These results are in agreement with other authors [5, 20]. For example, Gokmen *et al.* reported that custom membranes caused a notable decolorization in both laccase-treated and control samples of apple juice (reduction in color varied from 28% to 45%) [20]. The color of the samples was increased by 90%

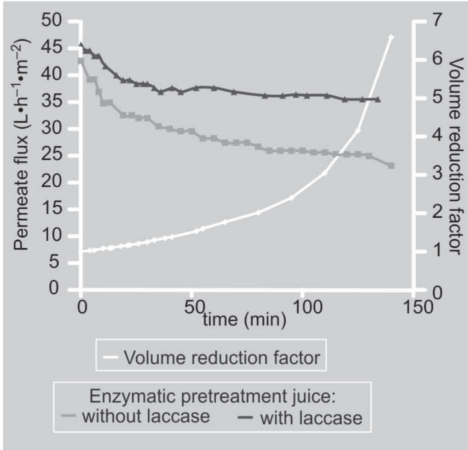


Figure 3
Variation of the permeate flux and volume reduction factor (VRF) with time for pomegranate juice treated by ultrafiltration at transmembrane pressure (TMP) = 2 bar and tangential velocity (V_m) = 1 $m \cdot s^{-1}$.

Table IV.
Characteristics of different pomegranate juices treated by enzymatic treatment or by ultrafiltration [transmembrane pressure (TMP) = 2 bar; tangential velocity (V) = 1 $m \cdot s^{-1}$].

Treated pomegranate juice	Total phenolic rejection	Color increase	Color reduction	Clarity decrease	Clarity improvement
			(%)		
Ultrafiltered	21	–	35	–	15
Laccase-treated	38	90[1]	–	166[1]	–
Laccase-UF-treated	49	–	38	–	32

[1] After laccase treatment, the absorbance increases at 420 nm for the color and at 650 nm for the clarity.

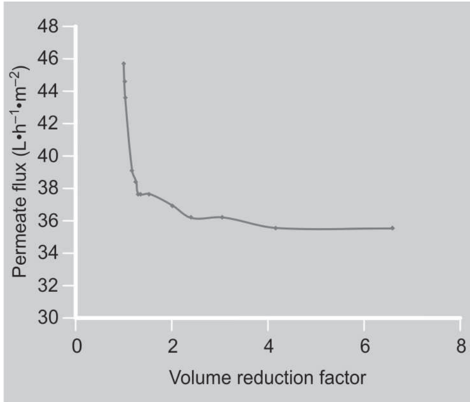


Figure 4
Effect of the volume reduction factor (VRF) on the permeate flux of pre-treated pomegranate juice treated by ultrafiltration [transmembrane pressure (TMP) = 2 bar; temperature (T) = 20 °C; tangential velocity (V) = 1 m·s^{-1}].

after oxidation of pomegranate juice with the laccase. The same result was observed by Alper and Acar (color samples increased by 115.2%) [5]. The clarity value decreased during laccase treatment (116%). Laccase-treated and further ultrafiltered pomegranate juice showed a 32% increase in its clarity. Our results are in agreement with those of Gokmen *et al.* [20].

The permeate flux of UF, in batch concentration mode, of pre-treated pomegranate juice decreased gradually with the operating time by increasing the volume reduction factor (VRF) due to concentration, polarization and gel formation (*figure 3*). The initial permeate flux of 46 L·h^{-1}·m^{-2} decreased from 35 L·h^{-1}·m^{-2}, corresponding to a final VRF value of 6. The flux permeation *versus* the VRF curve (*figure 4*) was divided into three periods: firstly, the permeate flux decreases rapidly due to the concentration polarization. Secondly, the permeate flux decreases slightly up to a VRF equal to 4, which corresponds with the beginning of the fouling. The last period of the curve is characterized by a steady-state flux due to complete fouling. These observations corroborate the results obtained by Cassano *et al.* for clarification of kiwi fruit and blood orange juice [25, 35].

3.3. Fouling mechanism

The best prediction for the fouling mechanism was obtained for pomegranate juice without laccase in the gel layer (n = 0, $k = 0.025\times10^{-3}$, $R^2 = 0.9867$) (*table V*). On the other hand, complete pore blocking (n = 2, $k = 53\times10^{-3}$, $R^2 = 0.9745$) becomes the predominant fouling mechanism for pre-treatment of pomegranate juice. Very good agreement is observed between experimental and theoretical data (*figure 5*). On the basis of the results obtained, it can be concluded that the fouling mechanism is affected by the pre-treatment process, and the solute nature and size has an important role in the solute–membrane physicochemical interaction and, consequently, in the fouling mechanism. If the results are compared with those obtained by Mirsaeedghazi *et al.* [36], perfect agreement with the untreated juice is noted. Unlike after pre-treatment of pomegranate juice,

Table V.
Estimates of the model parameters k and n of pomegranate juice ultrafiltered with and without laccase (enzymatic pre-treatment).

n	$k \times 10^3$	Measures of fit R^2 (ultrafiltered)	Measures of fit R^2 (laccase-UF-treated)
Complete pore blocking (n = 2)	29	0.9732	0.9259
	53	0.9453	0.9745
Internal pore blocking (n = 1.5)	5	0.9796	0.9430
	8	0.9747	0.9626
Partial pore blocking (n = 1)	0.6	0.9724	0.9082
	1.5	0.9688	0.9594
Gel layer (n = 0)	0.025	0.9867	0.9697
	0.020	0.9809	0.9544

the fouling mechanism can change during the filtration process due to the elimination of large particles by the pre-treatment [36].

4. Conclusions

Pomegranate juice was clarified by cross-flow ultrafiltration (UF) using a tubular mineral membrane with a molecular weight cut-off of 15 kDa. The effect of transmembrane pressure (TMP) and enzymatic pre-treatment on the permeate flux and quality of clarified juice was investigated.

In the operating conditions (2 bar and 1 m·s^{-1}), an average permeation flux of 36 L·m^{-2}·h^{-1} was obtained. Approximately 21% of polyphenolic compounds were retained; the initial clarity and color values of the raw pomegranate juice were, respectively, improved by 55% and reduced by 42%. UF application of clarified pomegranate juice decreased the amount of phenolic compounds that cause astringency and bitterness, thus improving clarity but reducing the natural red color.

The pre-treatment improved the permeation flux. The combined laccase-UF process improved the rejection of polyphenols and clarity. However, laccase-UF-treated and only UF-treated pomegranate juice showed the same decrease in their color.

The evaluation of the fouling mechanism of ultrafiltration of pomegranate juice showed that cake formation and complete pore blocking are the main mechanisms responsible for membrane fouling, respectively, for pomegranate juice without and with enzyme pre-treatment. This evaluation can help to find the process and methods of membrane regeneration. The membrane permeability can be regained after following a proper cleaning procedure. For example, cake formation is a reversible fouling mechanism; it can be removed easily by washing with water. On the contrary, complete blocking is not a reversible fouling mechanism, though rinsing with strongly alkaline or acidic agents at elevated temperatures may help to remove fouling.

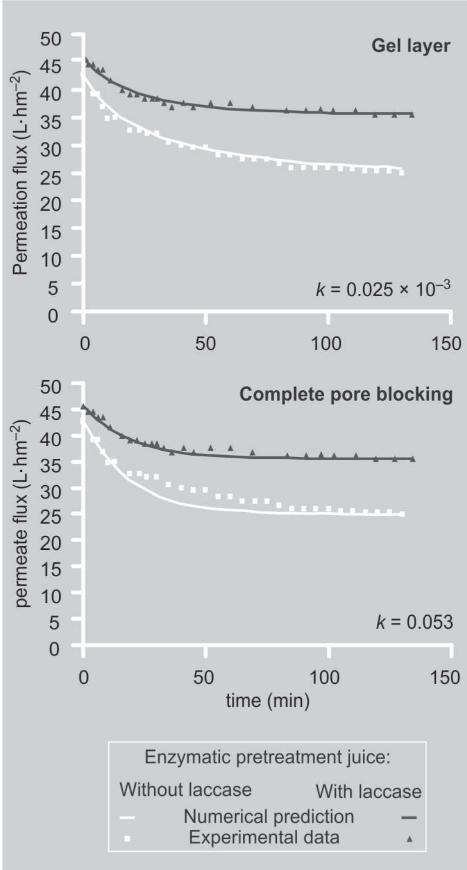


Figure 5
Gel layer formation model (n = 0; k = 0.025 × 10^{-3}) and complete pore blocking (n = 2; k = 0.053) for cross-flow filtration predictions from pomegranate juice treated with or without laccase.

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Clarificación del jugo granada por ultrafiltración: estudio de la calidad del jugo y del mecanismo de colmatación.

Resumen — **Introducción**. La ultrafiltración (UF) es un proceso simple para la clarificación y el afinamiento de jugos de frutas. Su objetivo consiste en eliminar las materias en suspensión, así como las sustancias que inducen enturbiamiento y turbidez durante su almacenaje. Concretamente, la polimerización de los compuestos fenólicos y su interacción con otros componentes (proteínas, por ejemplo) podrían causar enturbiamiento y turbidez en los jugos de fruta, dando lugar a la colmatación de la membrana de ultrafiltración. **Material y métodos**. Se clarificó un jugo fresco de granada por ultrafiltración. Los ensayos experimentales se efectuaron a modo de reciclaje total o por lotes, por reenvío del concentrado en la cuba de alimentación. Se estudiaron los efectos de la presión transmembrana (PTM), así como los de un pretratamiento enzimático en el flujo de permeación y en la calidad de los jugos. **Resultados**. Con el modo de reciclaje total, el efecto de la PTM en el color y en la claridad del jugo de granada clarificado fue significativo. El color del jugo de granada puro se redujo del 74% al 33% y su claridad disminuyó de un 77% a un 42%, cuando la PTM pasó de (1 a 3,6) bar. El desecho de los compuestos fenólicos disminuyó de un 45% a un 21% cuando la PTM pasó de (1 a 2) bar y se mantuvo constante por encima de 2 bar. En modalidad de tratamiento por lotes y con una PTM de 2 bar y una velocidad de 1 $m \cdot s^{-1}$, el tratamiento enzimático (5 $U \cdot mL^{-1}$, 300 min, T = 20 °C) del jugo de granada mejoró el flujo de permeado y dio lugar a una disminución de los compuestos fenólicos totales del 50% y a un aumento de la claridad del 30%. La colmatación de la membrana de UF durante el tratamiento del jugo de granada se debe principalmente a la retención de los polifenoles y/o de proteínas. En consecuencia, se han estudiado varios mecanismos de colmatación, empleando un modelo de colmatación de membrana desarrollado recientemente. El análisis reveló que el proceso de separación de la membrana estuvo controlado por el mecanismo de la capa de gel, para el jugo de granada puro, y por un mecanismo de bloqueo completo de los poros, para el jugo con pretratamiento enzimático.

Túnez / *Punica granatum* / jugo de frutas / clarificación / ultrafiltración / compuestos fenólicos / membrana / incrustación / procesamiento / lacasa