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Ultrafiltration for clarification of *Valencia* orange juice: comparison of two flat sheet membranes on quality of juice production

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Abstract

The aim of this study is to clarify of Moroccan Valencia orange juice by ultrafiltration (UF) using two flat sheet membranes characterized by different membrane materials (polyethersulfone (PES) and polysulfone (PS)) with molecular weight cut-off (MWCO) 30 and 20 kDa respectively. The performance of selected membranes was investigated in terms of selectivity and productivity towards total phenolic content (TPC), pectin content (AIS), total soluble solids (TSS),total flavonoids content (TFC) ,suspended solids (SS), ascorbic acid (AA), and total antioxidant activity (TAA). According to the results, both selected membranes allowed preserving of the original composition whereas the suspended solids and pectin content were completely removed. However, the PES membrane was the most suitable membrane for the clarification of the juice. In optimized operating conditions this membrane exhibited permeate flux of 71.14 L/m^{2}h and steady-state flux of 27.43 L/m^{2}h which was higher than PS membrane. Rejections towards TPC, AA, TFC and TAA for PES membrane were of the order of 10.1%, 6.2%, 11.32% and 9.85%, respectively. These values were lower than those determined for PS membrane.

1. Introduction

Membrane filtration application for the clarification of fruit juices has been extensively studied during the last decades. Membrane processes are very efficient in protecting the nutritional and sensory properties while obtaining high-quality, natural fresh-tasting and additive-free products as the separation process requires no heat application or the use of chemical agents [1]. Microfiltration, ultrafiltration (UF), nanofiltration and reverse osmosis are the main membrane processes [2, 3]. In particular, ultrafiltration (UF) represents a valid alternative to the conventional fining and filtration methods for clarifying fruit juice because this process can reduce enzyme consumption and eliminate fining agent and related problems, and moreover, it achieves continuous and reduces working time and simple processing [4-6]. This increases the yield in terms of the volume of clarified juice produced which is devoid of pectin and therefore, does not form a haze and has a reasonably longer shelf life. That is why; this process has gained popularity in the food industry in the last two decades [7-9].

Reports can be found in the literature on the treatment of various juices including kiwi fruit, mosambi, apple, orange, passion fruit, pineapple, and cactus pear. Permeate flux and product qualities are two important aspects during UF process. A high permeate flux is necessary for filtration to be practical and economic, and product quality should at least meet those obtained by the other standard clarification methods [10, 11]. The main problem in practical application of UF is the reduction in permeate flux with time, caused by the accumulation of feed components in the membrane pores and on the membrane surface [12-14].

It is well known that membrane material and molecular weight cut-off (MWCO) may influence the juice quality. In addition, operating parameters, such as transmembrane pressure (TMP), feed velocity and temperature, have a strong effect on the optimization of membrane performance in terms of maximization of permeate output and minimization of energy consumption. Indeed, one of the main problems of using membranes for clarifying juices is the decay of permeate flux due to membrane fouling which is attributed to the accumulation of macromolecular or colloidal species on the membrane surface. This phenomenon is known as concentration polarization, which leads to a rapid decrease of flux [15-17, 7]. This problem can be overcome by an enzymatic treatment of the juice, in which the colloidal particles are first degraded before the UF step, which is carried out by adding pectinases. It enables the reduction of the viscosity of the juice by depolymerization of insoluble pectin [18]. Although several studies indicate a positive effect on the permeate flux when TMP is raised, the use of higher TMP values leads to a more accentuated formation of fouling and polarized layers.

This study was aimed at evaluating the performance of two flat sheet UF membranes in the clarification of depectinized Moroccan Valencia orange juice. In particular, the experimental work was addressed to evaluate the influence of membrane material and molecular weight cut-off (MWCO) on the quality and content of antioxidant compounds of the clarified juice. For this purpose, two flat sheet membranes with different molecular weight cut-off (MWCO) (20 and 30 kDa) and membrane material (polysulfone and polyethersulfone) were used. The performance of each membrane in terms of permeate flux was also evaluated in optimized conditions of transmembrane pressure (TMP), temperature and axial feed flow rate (Qf).

2. Materials and methods

2.1. *Preparation of Valencia orange juice*

Valencia orange juice was prepared in laboratory from fresh fruits cultivated in Regional Agricultural Research Center in Kenitra, Morocco. Fruits were manually washed with water in order to remove surface dirt. Then, they were cut crosswise and squeezed by a domestic juicer. The squeezed juice was depectinized by using a commercial pectinase from *Aspergillus aculeatus* (Pectinex® Ultra SPL from Aspergillus Aculeatus, Sigma-Aldrich), which was added in a quantity of 20 mg/L. The enzyme is able to hydrolyze both high and low esterified pectins and also partially hydrolyze cellulose and hemicellulose [19]. The juice was incubated for 4 h at room temperature in plastic tanks and then filtered with a nylon cloth. The depectinized juice was stored at -20 °C and was defrosted to room temperature before the UF treatment.

2.2 *Ultrafiltration unit and procedures*

UF experiments were performed in a laboratory pilot cross-flow filtration unit supplied by Sterlitech Corporation (Sterlitech Corporation, WA, USA), equipped with a Sepa CF membrane Cell System Figure 1. Two different flat sheet membranes, with dimensions of 190 × 140 mm and an effective membrane surface area of 0.014 m^2, were used to clarify the depectinized juice. They were supplied by Sterlitech Corporation (WA, USA). Their characteristics are reported in Table 1. UF 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 operating conditions on the permeate flux. In this case permeate was continuously recycled to feed tank in order to ensure a steady state in the volume and composition of the feed. In the batch concentration mode in which permeate was continuously collected and the retentate stream were recirculated back to the feed tank, the UF system was operated at a TMP of 2 bar, at an axial feed flow rate (Qf) of 228 L/h and the temperature was maintained at 27°C, by cooling system (polyscience, USA). The permeate volume was collected in a measuring cylinder every 10 min to determine the permeate flux up to a volume reduction factor (VRF) of about 3 units; VRF is defined as the ratio between the initial feed volume and the final retentate volume, according to the following equation:

$$VRF = \frac{Vf}{Vr} = 1 + \frac{Vp}{Vr}$$

Where *Vf*, *Vr*, and *Vp* are the volume of feed, retentate, and permeate, respectively.
The conversion rate (Y) was calculated as follow:

$$Y = \frac{Qp}{Qf} \times 100$$

Where Qp is permeate flow, Qf is initial feed flow.

The hydraulic permeability of each membrane was determined by the slope of the straight lines obtained by plotting the water flux values in selected operating conditions versus the applied TMP.
The membrane filtration process can generally be described by Darcy's law as follow:

$$J = \frac{Q}{S} = \frac{TMP}{\mu Rm} = Lp * TMP$$

Where J ($L.m^{-2}.h^{-1}$) is the permeation flux, Q is permeate ($L.h^{-1}$) flow, S is surface of the membrane (m^2), TMP is the transmembrane pressure (bar), μ (Pa.s) is the viscosity of the permeate and Rm (m^{-1}) is the resistance to the permeate and Lp is permeability of membrane ($L.m^{-2}.h^{-1}.bar^{-1}$).
The rejection (R) of UF membranes towards specific compounds was calculated as follows

$$R = 100\left(1 - \frac{Cp}{Cf}\right)$$

Where Cp and Cf are the concentrations of specific component in the permeate and feed, respectively.

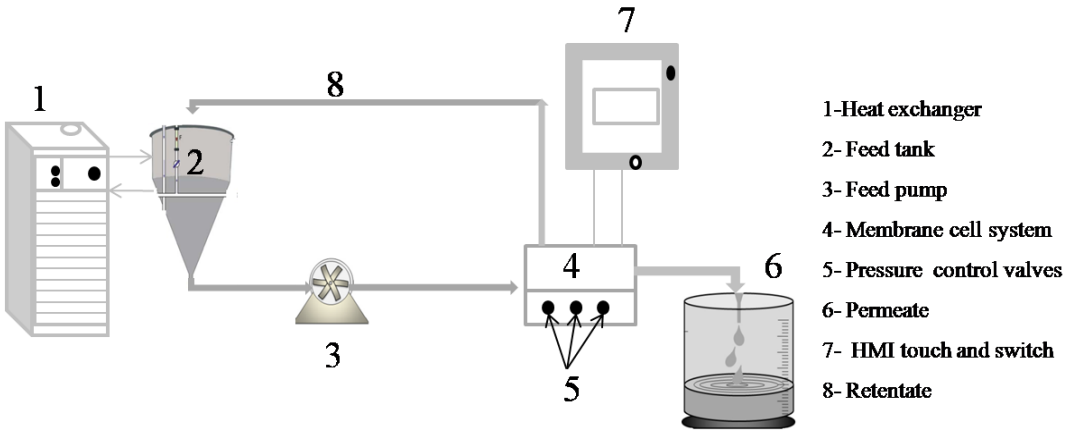

Figure 1: Scheme of UF laboratory pilot

Table 1: Characteristics of flat sheet ultrafiltration (UF) membranes.

Manufacturer	Designation	Membrane material	MWCO (kDa)	Operating pH
Nanostone	PS35	Polysulfone	20	1-10
Synder	MK	Polyethersulphone	30	1-11

2.3. *Analytical Methods*
2.3.1. *Analysis of Physico-chemical Properties*
Samples of fresh, clarified (permeate) and concentrated (retentate) juice coming from the UF experiments performed according to the batch concentration mode were collected and stored at -20°C for further analyses. The juice was analyzed for color, clarity, soluble solids, suspended solids content, pH, acidity, viscosity, density and pectin content.
Color and clarity of the juice were evaluated according to [20].They were evaluated by measuring the absorbance at 420 nm and transmittance at 660 nm, respectively, using a UV/Vis spectrophotometer **(**SPECORD® 210 PLUS, analyticjena, Germany**).** Total soluble solids (TSS) were measured, using a ATAGO digital refractometer (Atago Co., Ltd., Tokyo, Japan) and the results were expressed as °Brix. Acidity (TA) measurements were carried out by titrating 10 mL of the juice sample with 0.1 N NaOH until the solution pH reached 8.2 and expressed as wt % anhydrous citric acid equivalent. The pH values of the solutions were measured using a pH meter (Hanna Instruments., HI2221, USA) at 25°C. Viscosity was measured by using a FUNGILAB viscometer (Barcelona, Spain). The density of juice was determined using 25 ml juice by volumetric flask of 25 ml and precision balance. The suspended solids content (SS) was determined with the total juice relation (%w/w) by centrifuging according to [21], at 2000 rpm for 20 min, 45 mL of a pre-weighted sample; the weight of settled solids was determined after removing the supernatant.
The content of pectic materials was measured in terms of alcohol insoluble solids (AIS) according to [22]. AIS values were determined by boiling 20 g juice with 300 mL of 80% alcohol solution and simmering for 30 min.

The filtered residue was then again washed with 80% alcohol solution. The residue was dried at 100°C for 2 h and was expressed in percentage by weight.

2.3.2 *Determination of total flavonoids content (TFC)*
The total flavonoids content was spectrophotometercally determined by the aluminum chloride method based on the formation of complex flavonoid-aluminum according to [23]. 1 mL of dilute juice was mixed with 1 mL of $AlCl_3$ methanolic solution (2%w/v). After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm. The contents of TFC were estimated from the standard calibration curve of 4-40 mg/ mL quercetin.

2.3.3 *Determination of total phenolic content (TPC)*
Determination of total phenolic content was carried out according to [24]. 100 µL of dilute juice was dissolved in 1500 µL (1/10 dilution) of the Folin–Ciocalteu reagent. The solutions were mixed and incubated at room temperature for 1 minute. After 1 minute, 1500 µL of 75 g/L sodium carbonate (Na_2CO_3) solution was added. The final mixture was shaken and then incubated for 30 min in the dark at room temperature. The absorbances of all the samples were measured at 765 nm using UV-Vis spectrophotometer. Gallic acid was used as standard for curve calibration and was plotted at 0.03-0.42 mg/ml (Gallic acid that was prepared in 80% (v/v) methanol). The absorbance was recorded at 765 nm using 80% (v/v) methanol as blank. The estimation of total phenols was carried out in triplicate and the results were expressed in mg/L of gallic acid.

2.3.4 *Determination of ascorbic acid (AA)*
Ascorbic acid was determined by HPLC, according to [25]. HPLC performed by using a Jasco, PU 2089 plus separation module (Jasco, Japan) equipped with an UV-Vis detector. The analytical column was a 150 × 4.6 mm i.d., C18 Microsorb, thermostated at 25°C. The solvent system used was a gradient of solvent A (water with 0.1% v/v acetic acid) and solvent B (methanol). Samples of UF permeate were directly injected (after filtration on 0.45 µm HPLC filters),whereas feed juices and UF retentate were previously rediluted ,and then centrifuged at 5000 rmp for 15 min, in order to remove the pulp fractions. The following gradient was applied: 0-15 min, 5% B; 15-40 min, 80% B; 40-42 min, 5% B; and 42-50 min, 5% B. The flow rate was 0.9 mL min-1. HPLC filters and monitored at 278 nm. The concentration of ascorbic acid was calculated from the experimental peak area by analytical interpolation in a standard calibration curve and was expressed as mg/l of orange juice. Each assay was performed in triplicate.

2.3.5 *Total antioxidant activity (TAA)*
Evaluation of antioxidant activity of Valencia orange juices was measured by DPPH° radical (DPPH test) according to [26]. Briefly, the samples were diluted and centrifuged at 4000 rmp for 15 min. 2.5 mL of sample solution was added to 0.5 mL of 0.2 mM DPPH solution. The reaction mixture was shaken and kept for 30 min at room temperature in the dark. The absorbance of the solution was measured at 517 nm. The percentage inhibition was calculated according to the equation:

$$\text{Inhibition (\%)} = (A_c - A_s / A_c) \times 100.$$

Where A*c* is the absorbance of control (containing DPPH solution), As is the absorbance of sample. Antioxidant activity was expressed as mg Trolox equivalent/100ml of sample. All calculations were performed in triplicate.

3. Results and discussion

3.1. *Effect of operating conditions on permeate flux*
UF experiments were carried out according to the total recycle mode, were performed in order to study the effect of TMP, temperature and axial feed flow rate on the permeate fluxes.

3.1.1. *Effect of the TMP on the permeate flux*
Figure 2 shows the effect of TMP on the permeate flux values at steady state versus the applied TMP in selected operating conditions of feed flow rate (114 L/h) and temperature (20 °C) for both the investigated membranes. At low pressures the permeate flux resulted proportional to the applied pressure; a further increase in pressure did not improve the permeate flux and a limiting flux value was reached. According to the gel polarization model, the existence of a limiting flux is related to the polarization concentration phenomenon that arises as the feed solution is convected towards the membrane where the separation of suspended and soluble solids from the bulk solution takes place. The formation of a viscous and gelatinous-type layer is responsible for

an additional resistance to the permeate flux in addition to that of the membrane [27]. For both the investigated membranes a limiting flux was observed at an applied pressure of 2 bar. In the selected operating conditions found that, PES membrane exhibited higher fluxes when compared to PS membrane.

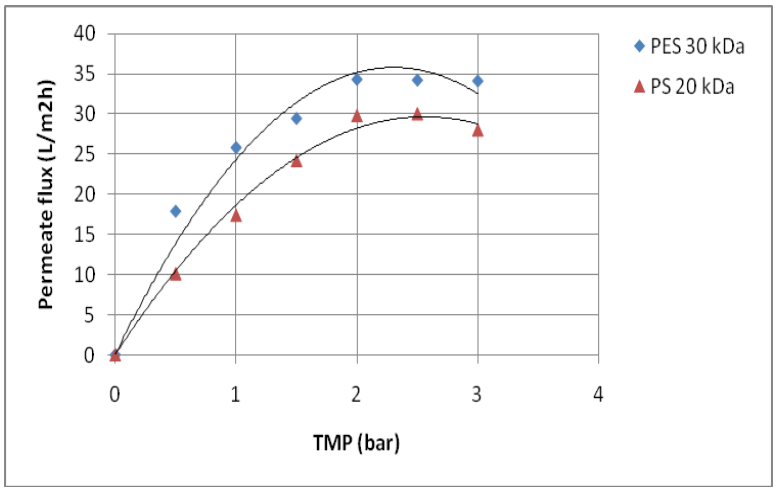

Figure 2: Effect of the TMP on the permeate flux (T = 20°C; Qf = 114 l/h).

3.1.2. *Effect of feed flow rate (Qf) on permeate flux*

Figure 3 shows the effect of axial feed flow rate (Qf) on the permeate flux in fixed conditions of TMP (2 bar) and temperature (20 °C). For both the investigated membranes an increase in the flow rate led to higher permeate fluxes. According to the film model an increase in the recirculation velocity improve hydrodynamical conditions, reduces concentration polarization, enhances the mass transfer coefficient, and increases the permeation flux [28].

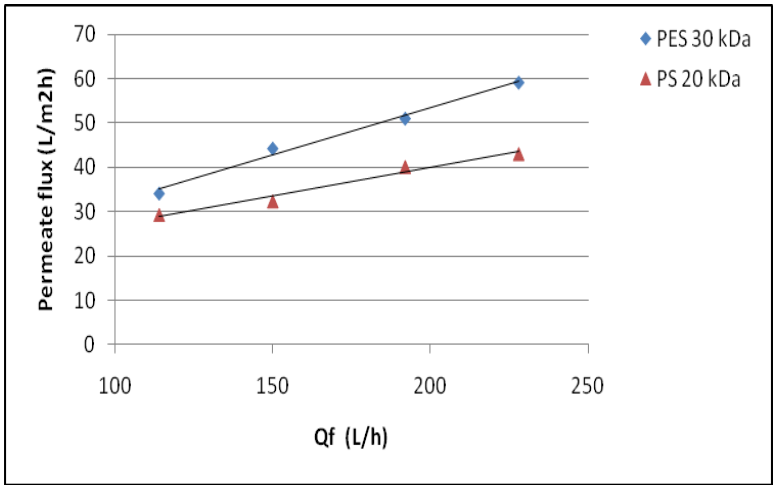

Figure 3: Effect of feed flow rate on the permeate flux (T = 20°C; TMP = 2 bar).

3.1.3 *Effect of temperature on the permeate flux*

The influence of temperature on the permeate flux is due its effect on solution viscosity, when the operating temperature is raised the feed viscosity is reduced and the diffusion coefficient of macromolecules increases. The effect of these two factors is to enhance the mass transfer and increase the permeation rate. For each increasing of 1°C the permeate flux increased approximately at a rate of 1.27 L/m2h (2.3%) for PS and 1.4 L/m2h (3.3%) for PES membrane.

3.2. *Batch concentration mode*

UF experiments carried out according to the batch concentration mode. Figure. 4 shows the time course of the permeate flux obtained in the UF treatment of the depectinized Valencia orange juice in selected operating conditions (TMP = 2 bar; feed flow rate = 228 l/h; temperature = 27 °C) for both the investigated membranes. The permeate flux (Jp) decreased gradually with the operating times due to concentration polarization and gel

formation. The initial permeate flux of 71.14 L/m^{2}h decreased to about 27.43 L/m^{2}h for PES 30 and 55.71 L/m^{2}h decreased to about 17.15 L/m^{2}h for PS membrane when the VRF value reached about 3. The Jp versus VRF curve (Figure. 5) 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 2, which corresponds to 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 [29-31] for clarification of kiwi, blood orange and apple juice.

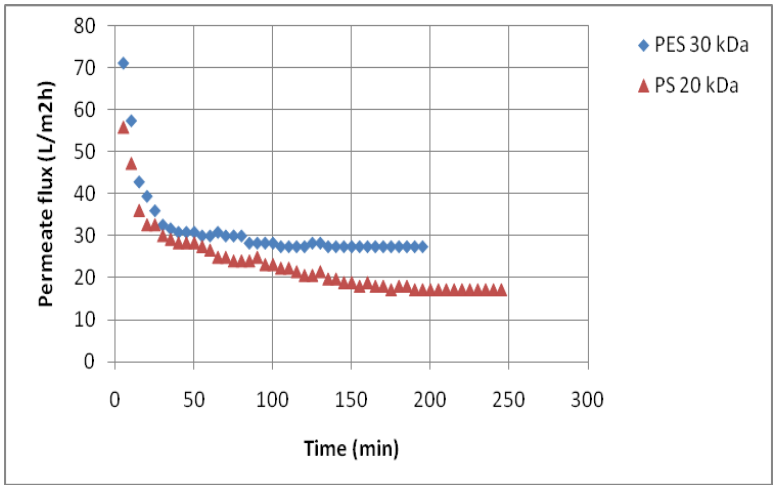

Figure 4: Time course of permeate flux (TMP = 2 bar; T = 27°C; Qf = 228 l/h).

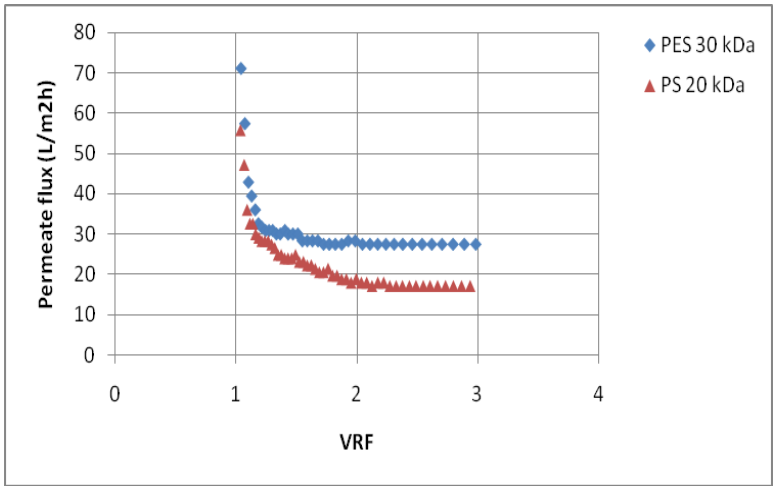

Figure 5: Effect of VRF on permeate flux (batch concentration mode; TMP =2 bar; Qf =228 L/h; T=27 °C).

3.3. *Effect of UF on chemical parameters of Valencia orange juice*

Table 2 shows the results of the analytical determinations performed on permeate retentate and feed samples coming from the UF treatment of the depectinized Valencia juice according to the batch concentration mode.

Suspended solids (SS) and the AIS were completely removed from the juice by the UF membranes and a clarified juice was obtained as permeate. There is an improvement in color and clarity of Valencia juice after filtration due to the removal of suspended colloidal particles present in juice. Lower cutoff membranes help to retain more colored compounds and haze precursors [10].

The TSS content of permeates decreased slightly with UF. In addition, TSS levels appeared to be higher in the retentate than in the permeate fraction: this phenomenon can be attributed to the presence of suspended solids content and soluble pectin in fruit juices that can interfere with the measurement of the refractive index. These observations corroborate the results obtained by several authors [21, 32-35]. The decrease in the TSS values were found correlating with the MWCO membrane as the removal of suspended solids increased with decreasing membrane MWCO; however, this relationship was not significant.

Table 2: Physicochemical characterization of depectinized Valencia orange juice submitted to UF treatment.

Characteristic	Feed	PES 30 kDa		PS 20 kDa	
		Permeate	retentate	permeate	retentate
Color (A_{420})	0.76	0.12	1.15	0.103	1.64
Clarity (%T_{660})	45.57	97.19	27.71	97.31	18.25
SS (W/W %)	4.12	0	6.01	0	6.01
TSS(°Brix)	11.09	10.86	11.95	10.84	12.11
Acidity (% CA)	1.02	1.01	1.02	0.99	1.04
pH	3.32	3.30	3.35	3.29	3.37
Density (g/cm^3)	1.08	1.03	1.1	1.02	1.1
Viscosity ($mpa.s^{-1}$)	1.45	1.04	1.95	1.03	1.94
AIS (Wt %)	0.19	0	-	0	-

The viscosity and Density of filtered juice have been reduced significantly due to the removal of all the suspended solids and pectic material during filtration, and they are close to water viscosity. Similar results were obtained by [36].

The pH and TA values were slightly changed with UF, but it can be said that UF and the different membrane MWCO did not have a significant effect on these values. Similar results are reported by other researchers as well [4, 12, 34, 37].

Table 3: Effect of flat sheet UF membranes on TFC, TPC, AA, and TAA of Valencia orange juice.

Membrane	sample	TPC (mg GAE/L)	TFC (mg QE/L)	AA (mg/L)	TAA (mgTE/100ml)
PES 30 kDa	Feed	649.05	249.15	474.16	29.96
	Permeate	583.49	220.94	445.36	27.01
	Retentate	741.6	290.88	419.13	35.64
PS 20 kDa	Permeate	568.57	215.72	437.53	25.91
	Retentate	769.13	298.91	431.2	37.02

GAE: gallic acid equivalent, QE: quercetin equivalent, TE: Trolox equivalent

Total phenolic content: Table 3 shows the effect of UF membrane on the total phenolic content. The TPC of clear Valencia orange juice ultrafiltered through the membrane PS was found to be the lowest compared to the membrane PES. The TPC levels of ultrafiltered Valencia orange juice through the PS and PES membranes were found to be 568.57 and 583.49 mg GAE/L, respectively. The reduction in TPC was more profound with decreasing MWCO membrane. This may have occurred because some polyphenols in Valencia orange juice are probably associated with other components which were rejected by the membranes with a smaller MWCO. UF through the PES membrane rejected 10.10%, while the PS membrane rejected 12.4% of total polyphenols (Table 4). Many researchers have found that there are positive relationships between membrane MWCO and TPC in UF applications, as a decrease in the MWCO membrane results in a decrease in the TPC of clear fruit juice [34, 35,37, 38]. But in this case the difference is very slight because the MWCO of two membranes are close.

TAA: the rejection of UF membranes towards TAA was about 9.85% for PES and 13.52% for PS (Table 4). In addition, a strong relationship was observed between the rejection of UF membranes towards phenolic compounds and the TAA rejection. These results can be attributed to the strong contribution of polyphenols to the TAA of the Valencia orange juice. In permeate of both membranes a little reduction of the TFC was observed in comparison with the feed (11.32% for PES and 13.41% for PS (Table 4).

Ascorbic acid: The AA content of Valencia orange juice decreased with UF, with a significant effect on the AA content, while the effect of MWCO membrane on the AA content was found to be non significant. This phenomenon can be explained on the basis of quite small molecular weight of this compound. Therefore, it can easily pass through the PES 30 kDa and PS 20 kDa membranes. The AA content of permeates obtained using the PES and PS membranes was found to be 445.36 and 437.53 mg/L, respectively. In the clarified juice, the

reductions in AA were found to be 6.2% (for PES) and 7.86% (for PS) in the same order with respect to feed juice. In Table 5 the mass balance of the UF process for ascorbic acid, TAA, total phenols content and flavonoids is reported. This balance is referred to an UF run in which, starting from 2 L of depectinized juice, VRF about 3, recovery factor = 66.5%) were obtained. It can be noted that the recovery of investigated compounds in the permeate of the process was higher than 58 % for PES membrane and 57% for PS membrane. The loss of AA was 8.06 % for PES membrane while was 8.31% for PS membrane, these reductions of AA, as quantified by the mass balance, could be due to oxidation of this component caused by continual recycling of the juice around the UF system, an interaction solute–membrane, and consequent adsorption of solute on the membrane surface or inside the pore, can be also considered. Cassano [30] reported that the reduction of AA in clear blood orange juice was 18.1% with the 15 kDa tubular PVDF membranes, while Toker [34] found to be 18.3, 19.59 and 20.42% in blood orange juice with 100, 50 and 30 kDa PES membranes respectively and Cassano [2] found this reduction to be 16% in kiwi fruit juice.

Table 4: Rejection of UF membranes towards TSS, TFC, TAA, AA, and TPC of Valencia orange juice.

	Rejection (%)				
Membrane	**TPC**	**TSS**	**TFC**	**AA**	**TAA**
PES 30 kDa	10.10	2.07	11.32	6.2	9.85
PS 20 kDa	12.4	2.25	13.41	7.86	13.52

Table 5: Mass balance of the UF process

		PES 30 kDa					PS 20 kDa				
Parameters	Feed	Permeate		retentate		Balance	permeate		retentate		Balance
Volume(L)	2	1.33	66.5%	0.67	33.5%	100%	1.33	66.5%	0.67	33.5%	100%
AA (g)	0.95	0.59	62.37%	0.28	29.57%	91.94%	0.58	60.72%	0.29	30.97%	91.69%
TPC (g)	1.3	0.78	59.78%	0.49	38.28%	98.06%	0.75	57.73%	0.53	40.41%	98.14%
TFC (g)	0.5	0.29	58.97%	0.20	39.11%	98.08%	0.28	57.08%	0.20	40.93%	98.0%
TAA (g)	0.6	0.35	58.54%	0.20	39.11%	98.62%	0.34	57.04%	0.25	42.13%	99.17%

Conclusion

The clarification of Moroccan Valencia orange juice was studied by ultrafiltration (UF) using flat sheet membranes with different membrane materials (polyethersulfone (PES) and polysulfone (PS)) and with molecular weight cut-off (MWCO) 30 and 20 kDa respectively. The performance of selected membranes has been investigated in terms of productivity and selectivity towards the compounds contributing to the quality of the juice. In optimized operating conditions of transmembrane pressure, temperature and feed flow rate, PES membrane exhibited permeate flux of 71.14 L/m^{2}h which was higher than permeate flux (55.71 L/m^{2}h) related to PS membrane. According to the results, both selected membranes allowed preserve of the original composition of the Moroccan Valencia orange juice in terms of antioxidant compounds content. On the basis of permeate flux data and chemical composition of the clarified juice, the most suitable membrane for the clarification of the juice was found to be the PES membrane. Therefore, the clarification process of the juice based on the exclusive use of membrane filtration was found to be an effective method for clear Valencia orange juice production with high quality attributes.

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