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Study of effect of clarification by ultrafiltration using flat sheet membrane on Quality of Valencia orange juice

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ABSTRACT

The clarification of Moroccan Valencia orange juice performed by ultrafiltration (UF) using flat sheet polysulfone (PS) membrane with molecular weight cut off 20 kDa. The freshly squeezed juice, after a depectinization step, was submitted to an UF process. In experimental tests performed according to the total recycle mode for study the effect of the operating conditions on the permeate flux and quality. The clarified juice was produced according to the batch concentration mode working in optimal operating and fluid dynamic conditions. The quality of the samples coming from the UF process was evaluated in terms of: total soluble solids (TSS), color, clarity, total antioxidant activity (TAA), total flavonoids content (TFC), ascorbic acid (AA), and total phenolics content (TPC). The clarified orange juice was highly close to the initial juice except for suspended solids (SS) and pectin content which were totally concentrated in the retentate. In the permeate of the UF process a low reduction of TPC (12.4%), TAA (13.52%), TFC (13.41%) and AA (7.86%) were observed with respect to the initial juice.

Key words: Ultrafiltration, Clarification, Valencia orange juice, Flat sheet membrane, Quality.

INTRODUCTION

Fruit and vegetable juices are beverages of high nutritional value. These beverages possess several key nutritional components such as minerals, vitamins and antioxidants. Among several beverage processing sectors, citrus fruits constituting orange, lemon, pineapple and mosambi. Orange juice is probably the best known and most widespread fruit juice all over the world, particularly appreciated for its fresh flavor and considered of high beneficial value for its high content of vitamin C and natural antioxidants, such as flavonoids and phenylpropanoid [1, 2,3]. Citrus fruits primarily constitute both lower-molecular-weight compounds (such as sugar, acid, salt, flavor, aroma compounds, etc.) as well as higher-molecular-weight polysaccharides (such as pectic material cellulose, hemicellulose, etc.). Pectins are long-chain polysaccharides that cause cloudiness and post-bottling haze formation as well as their fermentation during long storage and also make the juice highly viscous, which can affect the shelf life and pose difficulties for subsequent processing [4, 5].

Traditional methods for juice processing involve filtration using fining agents to remove suspended and colloidal particles and low-pressure evaporation [6]. They are also characterized by high energy consumption for temperature

control and the use of large amounts of coadiuvants and additives (bentonite, gelatines, *etc.*) with consequent problems of environmental impact due to their disposal [7]. Unfortunately, during these processing steps, a major portion of the compounds that contribute toward the quality of the beverage (such as aroma, flavor compounds, sugar content and acidity) get deteriorated due to thermal and chemical treatment steps [8].

Membrane technologies can work as well or better than the existing technology regarding product quality, energy consumption and environmental issues in the agro-food industry [9].

Moreover, membrane processes are very efficient systems to protect 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 [10]. Microfiltration, ultrafiltration (UF), nanofiltration and reverse osmosis are the main membrane processes [11, 12]. Among these, UF membranes have been shown to be of potential interest for clarification of fruit juices and have become a commercial success. Advantages of the UF over conventional fruit juice processing are in terms of: increased juice yield; possibility of operating in a single step reducing working times; possibility of avoiding the use of gelatines, adsorbents and other filtration aids; reduction in enzyme utilization; easy cleaning and maintenance of the equipment; reduction of waste products; elimination of needs for pasteurization [13]. 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 other standard clarification methods [14, 15]. 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 [16, 17, 18, 19, 20]. This problem can be overcome by an enzymatic treatment of the juice; this treatment is carried out by adding enzyme pectinase. It enables the reduction of the viscosity of the juice by depolymerization of insoluble pectin [21].

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 Centre in Kenitra, Morocco. Fruits were manually washed with water in order to remove surface dirt. Then, they were cutting crosswise and then 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 [22]. 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 laboratory pilot cross-flow filtration unit supplied by Sterlitech Corporation (Sterlitech Corporation, WA, USA), equipped with a Sepa CF membrane Cell System Fig 1. The juice was ultrafiltered through flat sheet PS membrane with MWCO 20 kDa having an effective membrane area of 0.014 m^2 (PS35, Nanostone). It was supplied by Sterlitech Corporation (WA, USA). 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 (Jp). In this case 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 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 at a temperature of 27°C to clarify the juice up to a volume reduction factor (VRF) of about 3 units. The permeate volume was collected in a measuring cylinder every 10 min to determine the permeate flux, and then stored at -20 °C for further analyses.

The membrane permeability was determined from the slope of distilled water flux versus pressure.

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 rejection (R) of UF membranes towards specific compounds was calculated as follows:

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

Where *Cp* and *Cf* are the concentration of a specific component in the permeate and feed, respectively.

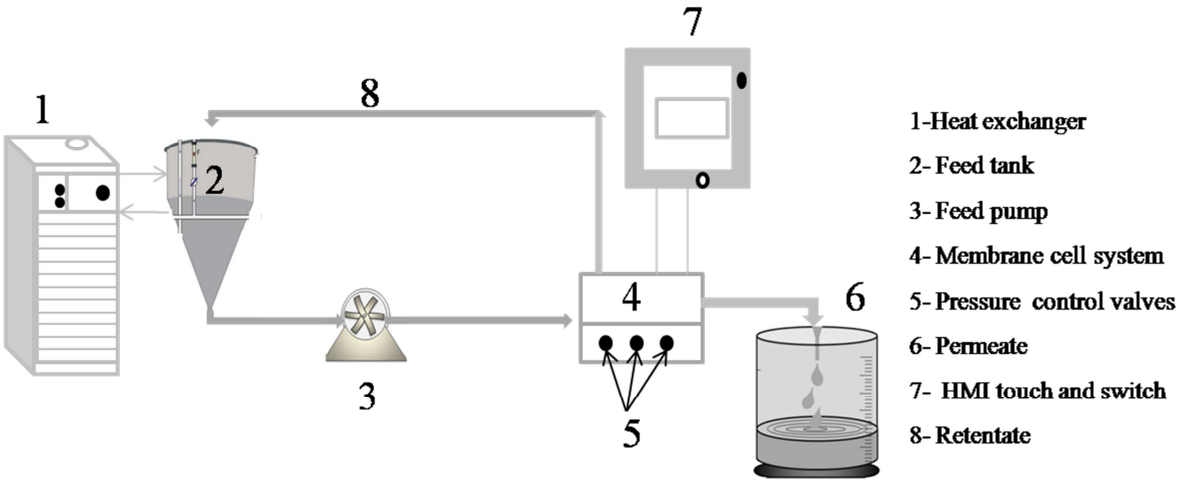

Fig 1: Scheme of UF laboratory pilot

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 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 [23].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 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, HI 2221, USA). **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 in relation to the total juice (w/w %) by centrifuge (UNIVERSAL 320R, Germany), at 2000 rpm for 20 min, 45 mL of a pre-weighted sample; the weight of settled solids was determined after removing the supernatant [24].

The content of pectic materials was measured in terms of alcohol insoluble solids (**AIS**) according to [25]. 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 flavonoids content (TFC)

The total flavonoid content was spectrophotometercally determined by the aluminum chloride method based on the formation of complex flavonoid-aluminum [26]. 1 ml of juice dilute 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 [27]. 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 absorbance of all samples was measured at 765 nm using UV-Vis spectrophotometer. Gallic acid was used as standard for the calibration curve and was plotted at (0.03-0.42) mg/ml Gallic acid that was prepared in 80% (v/v) methanol. The estimation of total phenols was carried out in triplicate and results were expressed as mg/L gallic acid.

2.3.4. Determination of ascorbic acid (AA)
Ascorbic acid was determined by HPLC, according to [28]. 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 permeate UF 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 [3]. 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:

$$\text{Inhibition (\%)} = (Ac - As / Ac) \times 100.$$

Where *Ac* is the absorbance of control (containing DPPH solution), *As* is the absorbance of sample. Antioxidant activity was expressed as mg Trolox equivalent/L of sample. All determinations were performed in triplicate.

RESULTS AND DISCUSSION

3.1. Effect of operating parameters on the permeate flux
UF experiments, 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
Fig. 2 shows 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 low pressures the solvent flux is proportional to the applied pressure. As the pressure is increased flux shows a deviation from a linear flux– pressure behavior and it becomes independent of pressure. In these conditions a limiting flux is reached at a TMP value of about 2 bar and any further pressure increase determines no significant increase of the permeate flux. The existence of a limiting flux can be related to the concentration polarization phenomenon that arises as the feed solution is convected towards the membrane where the separation of suspended and soluble solids from bulk solution takes place. A concentration profile from bulk solution to membrane surface is generated by the rejected material accumulated on the membrane. 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. The TMP limiting value (TMP_{lim}) depends on physical properties of the suspension and feed flow rate [29, 30]

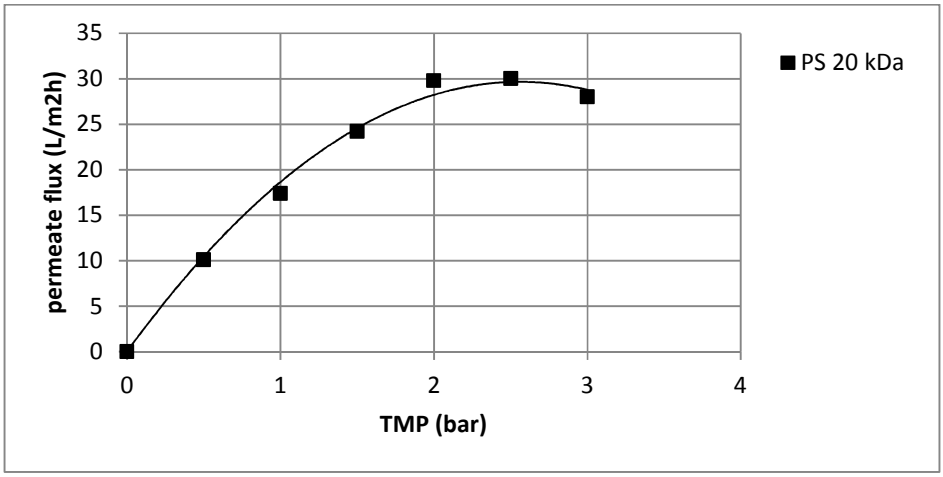

Fig. 2. Effect of the TMP on the permeate flux (T = 20°C; Qf = 114 l/h)

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

Fig. 3 shows the influence of the axial feed flow rate on the permeate flux at a temperature of 20°C and at a TMP of 2 bar: It was observed that the permeate flux increased linearly with increasing cross-flow velocity. The permeate flux increased from 29.23 to 43.11 L/m^{2}h while the feed flow rate increased from 114 to 228 L/h. Increasing of cross-flow velocity would enhance wall shear stress on the membrane surface. Higher wall shear stress is helpful to reduce concentration polarization and reversible fouling on the membrane surface [31].

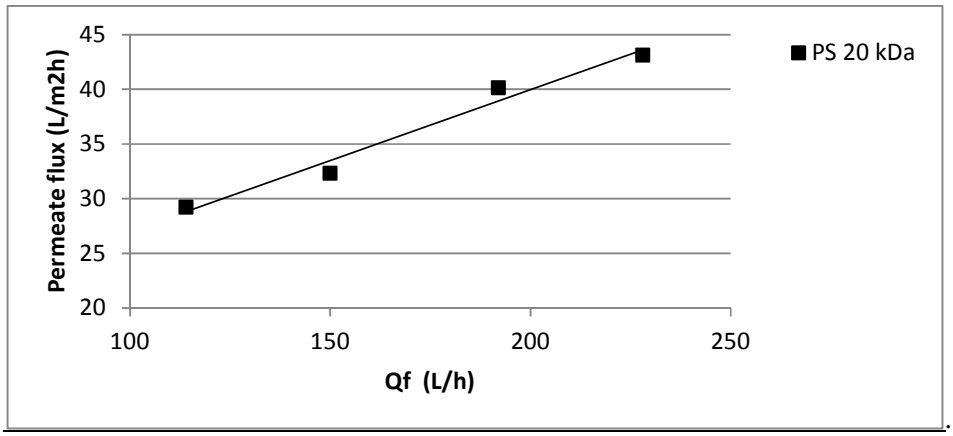

Fig.3. Effect of the axial feed flow rate on the permeate flux (T = 20°C; TMP = 2bar)

3.1.3. Effect of the temperature on the permeate flux

The influence of the temperature on the permeate fluxes: 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 mass transfer and to increase the permeation rate. For each increasing of 1°C the permeate flux increased approximately at a rate of 1.41 l/m^{2}h.

3.2. Batch concentration mode

UF experiments carried out according to the batch concentration mode were performed at a TMP of 2 bar, an axial feed flow rate of 228 L/h and a temperature of 27 °C. Fig. 4 shows the time course of the permeate flux obtained in the UF treatment of the depectinized Valencia orange juice. The permeate flux decreased gradually with the operating times due to concentration polarization and gel formation. The initial permeate flux of 55.71 l/m2h decreased to about 17.15 L/m^{2}h when the VRF value reached about 3. The Jp versus VRF curve (Fig. 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 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 [7, 29] for clarification of blood orange juice.

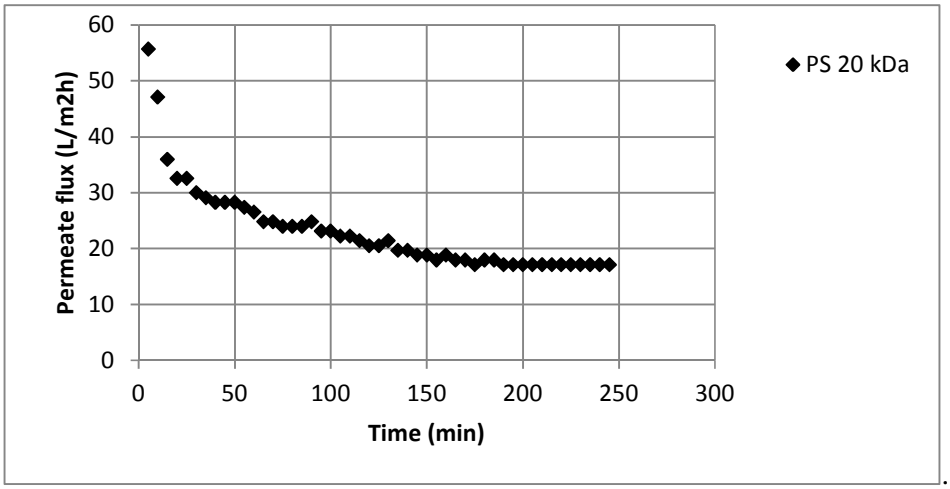

Fig. 4. Time course of permeate flux (batch concentration mode TMP = 2 bar; T = 27°C; Qf = 228 l/h)

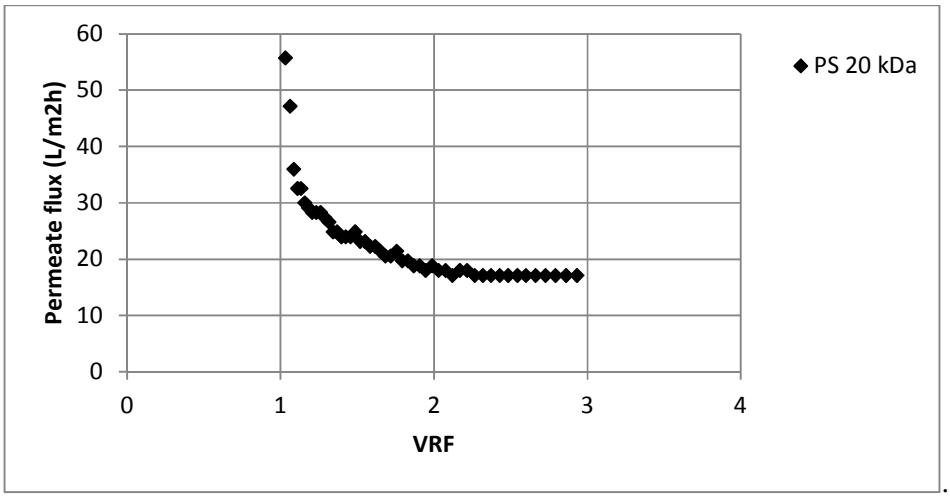

Fig. 5. Effect of VRF on permeate flux (batch concentration mode, TMP=2 bar; Qf=228 L/h; T=27 °C)

3.3. Analytical evaluations

Table 1 shows the results of the physico-chemical determinations performed on feed, permeate and retentate samples coming from the UF treatment of the depectinized Valencia juice according to the batch concentration mode.

AIS and **SS** were totally removed from the juice and a clarified juice was obtained as permeate. There is improvement in **color** and **clarity** of Valencia juice after filtration due to removal of suspended colloidal particles present in juice.

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 [7, 32, 33].

The **viscosity** and **density** of filtered juice have been reduced significantly due to removal of all the suspended solids and pectic material during filtration, and they are close to water viscosity, similar results were obtained by [34]. The **pH** and **TA** values were slightly changed with UF.

Table 1 Physicochemical characterization of depectinized Valencia orange juice submitted to UF treatment

Parameters	Color (A_{420})	Clarity ($\%T_{660}$)	TSS (°Brix)	SS Wt%	pH	Acidity %CA	Density g/cm^3	Viscosity $mpa.s^{-1}$	AIS (wt%)
Feed	0.76	45.57	11.09	4.12	3.32	1.02	1.09	1.45	0.18
Permeate	0.1026	98.31	10.84	0	3.29	0.99	1.02	1.03	0
Retentate	1.64	18.25	12.11	6.01	3.37	1.04	1.1	1.95	-

TPC Table 2 shows the effect of UF membrane on total phenolic content, the TPC of permeates was found to be 568.57 mg/L. The rejection of UF membrane towards TPC was 12.4% (Table 3). The reduction of TPC in permeate, can be attributed to some polyphenols in Valencia orange juice are probably associated with other components which were rejected by the membranes this agrees with results [32, 24, 35] . The rejection of UF membranes towards **TAA** was about 13.52% (Table 3). 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 the permeate of membrane a little reduction of the **TFC** was observed in comparison with the feed 13.41% (Table 3).

Table 2. Effect of UF on TFC, TPC, AA, and TAA of Valencia orange juice

Parameters	TPC (mg GAE/L)	TFC (mg QE/L)	AA (mg/L)	TAA (mgTE/100ml)
Feed	649.05	249.15	474.84	29.96
Permeate	568.57	215.72	437.53	25.91
Retentate	769.13	298.91	431.2	37.02

GAE:Gallic acid equivalent, QE: Quercin equivalent, TE: Trolox equivalent

Ascorbic acid: In the clarified juice a 7.86% reduction of the ascorbic acid was observed with respect the feed juice. In Table 4 the mass balance of the UF process for ascorbic acid, TAA, total phenols content and flavanoids is reported. This balance is referred to an UF run in which, starting from 2 L of depectinized juice, 1.318 L of permeate and 0.682 L of retentate (final VRF = 2.93, recovery factor = 65.9%) were obtained. It can be noted that the recovery of investigated compounds in the permeate of the process was higher than 57%. The 8.31 % loss of ascorbic acid, as quantified by the mass balance, was probably due to an 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 [29] reported that the reduction of AA in clear blood orange juice was 8.41% with the 15 kDa tubular PVDF membrane, while Toker [32] 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 [11] found this reduction to be 16% in kiwi fruit juice.

Table 3. Rejection of UF membrane towards TSS, SS, AIS, TFC, TAA, AA, and TPC of Valencia orange juice

Characteristic	TSS	SS	AIS	TPC	TFC	AA	DPPH
Rejection%	2.25	100	100	12.4	13.41	7.86	13.52

Table. 4 Mass balance of the UF process

	Feed	Total permeate		Final retentate		Balance
Volume(L)	2	1.318	65.9%	0.682	34.1%	100%
AA (g)	0.950	0.5767	60.72%	0.294	30.97%	91.69%
TPC (g)	1.298	0.749	57.73%	0.525	40.41%	98.14%
TFC (g)	0.4981	0.284	57.08%	0.204	40.93%	98.00%
DPPH (g)	0.599	0.342	57.04%	0.252	42.13%	99.17%

CONCLUSION

Moroccan Valencia orange juice was clarified by cross-flow ultrafiltration (UF) using a flat sheet polysulfone (PS) membrane with a molecular weight cut-off of 20 kDa. In the optimal operating conditions (2 bar, 228 l/h and 27°C) guaranteeing maximum permeation flux, minimum fouling and the clarified juice presents physico-chemical and

nutritional properties very close to those of the feed Valencia orange juice, except for the absence of suspended solids and pectin content which were totally concentrated in the retentate.

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