

Original Research Article

Extraction and concentration of *Cola nitida* compounds using membrane processes : analysis of operating parameters and membrane fouling.

ABSTRACT

Aims: Kola nut is a natural product rich in biological active compounds. This work was aimed at evaluating the potential of an integrated system based on the combination of membrane processes for the purification and concentration of kola extract bioactive compounds.

Place and Duration of Study: Mature kola seeds were collected in October 2014-February 2015 in South of Côte d'Ivoire and the membranes performances were carried out from May to November 2016 at the European Institute of Membranes, France.

Methodology: Kola extract obtained by ethanol-water mixture were clarified by Microfiltration (0.2 µm) and Ultrafiltration (5, 15 and 50 kDa). The choice of ultrafiltration membrane was carried out on a pseudo-tangential pilot and membranes chosen are characterized and validated on a tangential filtration pilot before the concentration of the extracts by nanofiltration (200-400 Da). The effect of crossflow velocity and transmembrane pressure was performed in recycle mode for each membrane. Fractions coming from the membrane processes were analyzed for their content in total polyphenols, proteins, caffeine, catechin and epicatechin.

Results: Microfiltration (0.2 µm) coupled with ultrafiltration (50 kDa) produced the purified extract and was submitted to a nanofiltration process to produce a concentrated fraction enriched in active compounds such as polyphenols and caffeine. Permeate flux significantly decreased with time until a steady-state was established due to the membrane fouling. The high retention of proteins in microfiltration (35.71%) and ultrafiltration (35.29%) favors a purity gain of the compounds of interest such as total polyphenols, caffeine and catechin. However, there is a loss of total polyphenols during ultrafiltration step due to its high retention (29.17%). This result in a low concentration factor in the final retentate (2.56) during the coupling of microfiltration/ultrafiltration/nanofiltration compared to that observed during the direct concentration of the crude extract (4.27). The opposite is observed for caffeine, catechin and epicatechin.

Conclusion: The membrane processes favor the purification and concentration of the bioactive compounds of *Cola nitida* nuts. However, the ultrafiltration step has the disadvantage of retaining a high proportion of polyphenols and therefore of reducing the antioxidant capacity of the final product.

Keywords : *Cola nitida*, kola extract, Microfiltration, ultrafiltration, nanofiltration, membrane fouling

1. INTRODUCTION

In the last decade there has been an increased interest in the extraction and separation of natural compounds with biological activity and potential benefits for human health [1-3]. Thus, the recovery of natural extract has been implemented successfully and has attracted considerable economical interest. Polyphenols are the most abundant antioxidants in human diet and the largest and best studied class of polyphenols is flavonoids, which include several thousand compounds [4-6]. As antioxidants, they protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated to oxidative stress [3,6]. The main dietary sources of polyphenols are fruits, vegetables, cereals, olive, dry legumes, chocolate and beverages such as tea, coffee and wine [1,4,5,7].

As coffee and tea, kola nut is a tropical seed which is important for its tonic and stimulating effect [8-10]. Kola nuts is greatly accepted among consumers to its high levels of phenolic compounds, such as flavonoids and phenolic acids; xanthines, such as caffeine and theobromine [9,11,12,13]. There

are also received great interest due to their pharmacological properties such as physical and intellectual stimulant, vomiting control, hunger and thirst counteracting effects [11,14,10,15]. For the formulation of foods and beverages enriched with antioxidant compound, these substances need to be extracted preferably from their natural sources and stabilized. Unfortunately, most of the technologies used (ultrasound-assisted extraction, heat treatment; enzyme-assisted extraction, supercritical fluid extraction, etc.) could cause the degradation of the compounds of interest due to high temperature and long extraction time [16].

Membrane processes offer several advantages in various sectors of production for their capacity to operate at room temperature under low energetic consumption when compared with conventional technologies for concentrating and/or fractionating bioactive compounds from different vegetables sources [16-17]. Particularly, microfiltration (MF) is generally used for fine particles or bacteria removal or turbidity reduction processes [18-22]. While, ultrafiltration (UF) is used for macromolecule concentration and fractionation [23-27]. The main applications of nanofiltration (NF) are water treatment, water demineralization and small organic molecules concentration [28-32]. As for reverse osmosis (RO), it is used for water desalination but also widely used for the concentration of plant extract or juice fruit [33]. Nevertheless, the combination of at least two pressure-driven membranes (MFT/RO, UF/NF, UF/RO, MF/UF, etc.) permits to achieve better separation [34-38].

This work was aimed at evaluating the potential of an integrated system based on the combination of membrane processes for the purification and concentration of kola extract bioactive compounds with desirable properties. In particular, kola extract obtained by ethanol-water mixture were clarified by microfiltration and ultrafiltration in order to remove macromolecular compounds and suspended solids. The purified UF permeate was then submitted to a nanofiltration process in order to obtain concentrated fractions of phenolic compounds and caffeine. A crude kola extract was also concentrated by nanofiltration in order to judge the efficiency of the previous coupling process. The appropriate ultrafiltration membrane was firstly selected on a pseudo-tangential filtration pilot by comparing the different membrane performances (flux, retention coefficient and volumetric reduction factor) during concentrate mode. All the selected membranes were characterized on a tangential filtration pilot close to industrial reality before submitting them to the final concentrate mode. The effect of crossflow velocity and transmembrane pressure was performed in recycle mode for each membrane in order to determine the optimal filtration conditions. Fractions coming from the membrane processes (MF, UF and NF) were analyzed for their content in total polyphenols, proteins, caffeine, catechin and epicatechin in order to evaluate the selectivity of each step toward compounds of interest. The performance of microfiltration, ultrafiltration and nanofiltration membranes was also evaluated in term of productivity in selected operating conditions. For that, the fluxes declines were evaluated at each step and the composition of retentates and permeates were assessed.

2. MATERIAL AND METHODS

2.1 Plant material and pre-treatments

Kola extract was extracted from mature kola seeds (*Cola nitida* Schott & Endl.) harvested from October to February 2015 in south of Côte d'Ivoire. Nuts were washed with distilled water, cut in smaller pieces and dried at room temperature ($30 \pm 2^\circ\text{C}$) during two weeks. The dried sample was milled into powder using an electric blender and stored in plastic bags before their achievement at Institut Européen de Membrane (IEM, Montpellier). The extract was obtained by infusion of 1 g of kola nut powder ($\varnothing$ 10 μm) in 100 mL of EtOH 50% for 24h at room temperature as described by Nyamien *et al.* (2014). The extract obtained was filtered through sintered glass (40 -100 μm) before use as crude extract (CE).

2.2 Chemical reagent

All reagents used in this study were of pure analytical grade, unless otherwise specified, were purchased from Sigma Aldrich (Germany): ethanol ($\text{CH}_3\text{CH}_2\text{OH}$), methanol (CH_3OH), hydrochloric acid (HCl), Folin-Ciocalteu's phenol reagent, sodium carbonate salt (Na_2CO_3), sodium hydroxide (NaOH), aluminium chloride (AlCl_3), Gallic acid, catechin, epicatechin, vanillin, sulfuric acid (H_2SO_4), nitric acid (HNO_3), sodium nitrite (NaNO_2), copper (II) sulfate (CuSO_4), potassium sodium tartrate ($\text{C}_4\text{H}_4\text{KNaO}_6$), serum albumin, caffeine, catechin and epicatechin.

2.3 Description of the pilot and membranes

The commercial MF, UF and NF flat sheet membranes used in this work were made of composite polyamide materials and ceramic materials (Table 1). In order to achieved the necessary crossflow velocity (CFV) and transmembrane pressure (TMP), two equipments with have been used :

- ✓ 1st- A semi-tangential filtration pilot composed of a SPIRLAB cell containing a ceramic plane membrane with an active area of 56.3 cm² for the rapid selection of filtration membranes and the implementation of a filtration process: (i)- The feed stream, or crude extract (1000 mL) of kola extract previously filtered under vacuum on filter paper (Wattman N° 1) was pumped continuously through the microfiltration unit (0.2 µm) by means of a peristaltic pump at a predetermined cross flow velocity (2.8 m/s) and transmembrane pressure (0.5 bar). The operational temperature was set and maintained at 25°C using a thermostatic bath. (ii)- An equal volume of permeate obtained from the microfiltration (permeate MF) was filtered through each ultrafiltration membranes (50, 15 and 5 kDa). For all tests, the transmembrane pressure was set at 2 bar and the recirculation rate maintained at 70 L.h⁻¹ (crossflow velocity of 2.8 m.s⁻¹). (iii)- Each ultrafiltration membrane was used for the filtration of 1000 mL of crude kola extract previously filtered under vacuum on filter paper (Wattman N°. 1). The operating conditions were the same as before.
- ✓ 2nd- A tangential filtration system on a pilot scale (Fig.1). It was possible to use different filtration modules on this pilot. All parts of the equipment were made of aqueous and organic solvent resistant materials and designed to operate at up to 69 bar and a maximum temperature of 177°C. The first was made of stainless steel, containing a ceramic tubular membrane (for MF and UF filtration) with an active surface of 101,2 cm² and the second module consisted of a SEPA CF Membrane Cell nanofiltration device (OSMONIC, Minnetonka, MN, USA) with an active surface of 155 cm². A variable speed Hydracell pump (able to work up to 60 bar with a maximum flow rate of 600 L/h) was employed, and the system was fitted with a flow meter. Pressure was monitored at the entrance and exit of the membrane module, and a needle valve located after the membrane module was used for TMP regulation. The temperature of the feed was assured by a thermostatic bath (Julabo, Germany). The feed temperature was checked by a manual thermometer. The system allows the use of a single membrane and therefore a separate handling of the permeate.

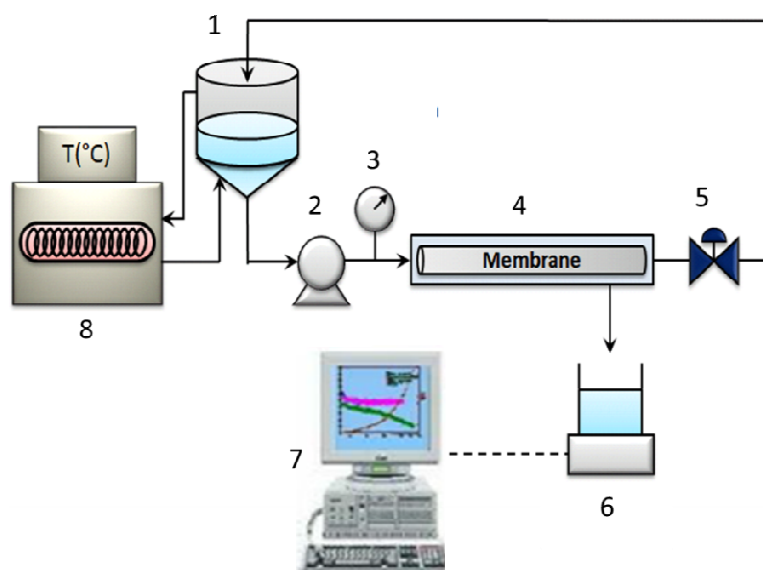


Fig.1 : Tangential filtration pilot (1) tank, (2) pump, (3) manometer, (4) membrane cell, (5) valve, (6) permeate, (7) computer, (8) thermostatic bath.

Table 1 : Characteristics of the membranes studied

	MWCO	Membrane /Type	Area of the membrane (cm ²)	Pro 	Pression maximum (bar)	EtOH 50% permeability (L/h m ²)*
Microfiltration	0.2 µm	Plane/tubular	56.5 / 101.2	Tami Industries	177	320 ^a
Ultrafiltration	50 kDa					146 ^b
	15 kDa					nd
	5 kDa					nd
Nanofiltration	200-400 Da	Plane	155	Filmtec Dow	45	38 ^c

*Determined in our laboratory at 25°C,

^a :determined at 1 bar ; ^b :determined at 2 bar ; ^c : determined at 10 bar

2.4 Filtration modes and experimental measurements

2.4.1 Full recycle mode

Full recycle experiments were carried out at different transmembrane pressure (TMP) and crossflow velocity (CFV) in order to determine the optimal operational conditions for each filtration step. The permeate flux J_p was measured in microfiltration and ultrafiltration at transmembrane pressure (TMP) in the range of 1 – 4 bar and 1 - 4 m/s for the velocity. For the nanofiltration, the range was 5 – 20 bar and 0,5 to 2 m/s for the pression and velocity, respectively. Successive experiments were carried out from lower to higher TPM at constant CFV. When the set maximum pressure value was achieved, the flow was measured, in the continuous, by reducing the pressure to its minimum value considered for the test to assess the membrane fouling. 30 min was given to permeate flux stabilized until flux become pressure or crossflow independant. In the case of velocity effect, the pressure was kept constant and the permeate flux were measured as before. The NF membrane was compacted [39] at 25 bar (until permeate flux stabilization) before the relaxation of the parameter considered.

2.4.2 Batch operation in concentration mode and calculation

Concentration experiments were carried out in batch mode, with simultaneous retentate recycling and permeate was collected in a beaker placed on an electronic balance (3100 g±0.01 g) Adventurer Pro AV3102C (OHAUS, China). For each step filtration, optimal conditions defined below were applied and feed stream was pumped from a temperature controller tank (25°C) through the membrane. Filtration tests were carried out in three steps:

- ✓ Solvent filtration to determine the initial permeability of the membrane L_{p0} . Permeate flux was obtained by measuring the volume permeating per unit filtration area per unit time and membrane permeability coefficient representing the liquid crossing the membrane per unit, per membrane area unit and per TPM unit. Experimentally, it is calculated by the slope of J_p vs TMP for the system :

$$J_p = L_p.TPM \quad (1)$$

- ✓ Filtration of the solution in batch mode : The apparent solute rejection coefficient (R) is defined as :

$$R = \left(1 - \frac{C_p}{C_f}\right) \times 100 \quad (2)$$

Where C_p and C_f are the concentration of the compound in permeate and feed stream, respectively

- ✓ Rinse with deionized water and measure the solvent permeability of the fouling membrane to determine L_{p1} : Permeability loss (irreversible fouling) was obtained from a comparison of the membrane water permeability (L_p) before and after the filtration:

$$\text{Permeability loss} = \frac{L_{p1} - L_{pf}}{L_{pi}} \times 100 \quad (3)$$

Where L_{pi} and L_{pf} are the solvent permeabilities of the new and fouled membranes, respectively.

Two experiments were carried out in parallel to evaluate the contribution of the various filtration steps on the extraction efficiency of the compounds and to evaluate the performance of the membranes. 1st- Initial volume of prefiltered kola extract (2000 mL) was charged into the feed vessel for the MF step. After pressurizing (1 bar) the system at the constant flow velocity of 2 m/s, permeate was collected separately (PMF). The volume rejection factor (VRF) was calculated as the ratio between the initial feed volume and the volume of remaining concentrate after the considered operation time :

$$VRF = \frac{V_f}{V_f - V_p} \quad (4)$$

Where V_f is the feed volume (L) and V_p the permeate volume (L).

The permeate collected was used as feed solution on UF membrane at the pressure of 3 bar and constant crossflow velocity (2 m/s). At the last time, UF permeate was concentrated by nanofiltration at TPM and velocity of 10 bar and 1 m/s, respectively. 2nd-On the other hand, crude extract was filtered directly through NF membrane in the same operating conditions (Fig.2).

According to the manufacturer instructions, a preliminary treatment of the nanofiltration is required before utilization. Thus, NF 270 membrane compaction was performed using pure solvent EtOH 50% at 25 bar until steady permeate flux is achieved. Samples of feed solution, permeate and retentate were collected at the end of each filtration for the quantification of total phenols, total sugars, proteins, caffeine, catechin and epicatechin. Membranes performances were evaluated with respect to two main parameters : permeate flux and rejection of certain target compounds (total phenols, total sugars, proteins, caffeine, catechin and epicatechin).

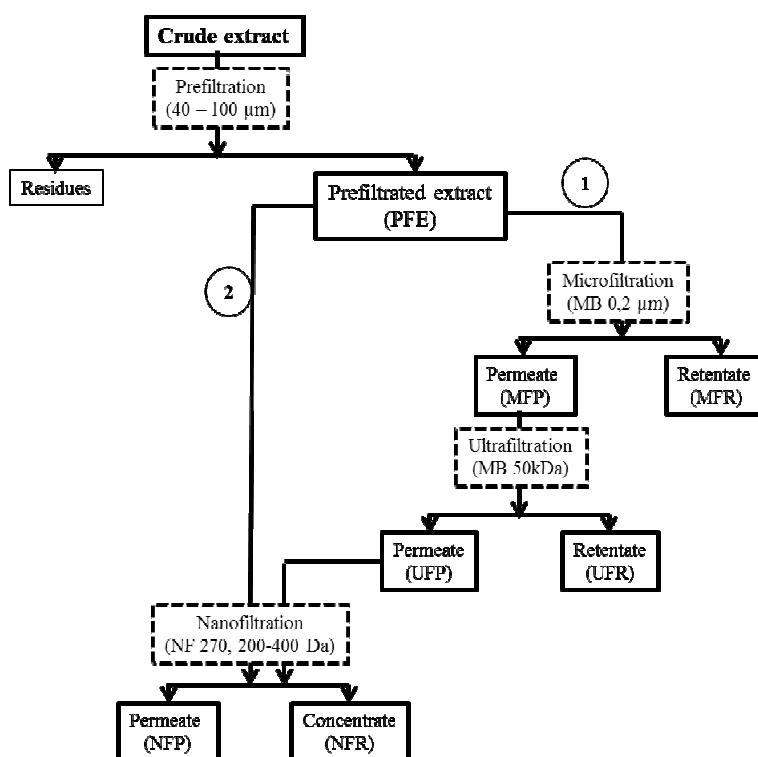


Fig.2 : Schematic presentation of the filtration protocol

2.4.3 Membranes cleaning

At the end of the experiments, the membranes are soaked in a solution of bleach (NaClO 2,6% of active chlorine) at 40°C for 15 min, rinse with deionised water and subjected to a chemical washing before their reuse. This washing is carried out according to a precise protocol and fabricant recommendations. The fouled membranes were cleaned in full recirculation by hydroxide sodium solution (NaOH 2%) at 80° C without pression for 15 min and 20 min at 2 bar, follows by nitric acid solution (HNO₃, 2%) at 60°C without pression for 10 min and 15 min at 2 bar. The system was operated with fresh water between the two steps until permeate is netral in order to check the flux recovery.

2.5 Analytical method

2.5.1 Total content of phenols

Total polyphenols were determined by colorimetry, using the Singleton and Rossi method [40] modified by Wood *et al.* [41]. Diluted Folin-Ciocalteu reagent (1/10, v/v, 2.5 mL) was added to 30 µL of sample. After 2 min of incubation in the dark at room temperature, 2 mL of aqueous sodium carbonate (75 g/L) was added. After gentle stirring, the mixture was incubated in a water bath at 50°C for 15 min and rapidly cooled down to stop the reaction. The absorbance was measured at 760 nm with distilled water as blank. A calibration curve was performed with gallic acid at different concentrations (0-1 g/L). Analyses were performed in triplicate and polyphenols level was expressed in grams of gallic acid equivalent per liter of extract (g/L GAE)

2.5.2 Total content of proteins

The proteins were determined according to the Lowry *et al.* [42] method after precipitation of the proteins with trichloroacetic acid (TCA). 200 µL of sample are pipetted into an ependorf tubes, and 500 µL of a diluted TCA (1/10) solution are added. After vortexing, the tubes are placed in a centrifuge for 15 min at 5000 rpm. Subsequently, the supernatant is aspirated by means of a water-jet tube and then the pellet neutralized. This neutralization is carried out by adding 200 µL of sodium hydroxide solution (0.2 N). The mixture is well stirred by vortexing and then incubated in a water bath at 60 °C. for 30 minutes. Finally, one milliliter of a solution C is added to the reaction medium. After vortexing and incubation at room temperature for 10 minutes, 0.1 mL of Folin Ciocalteu reagent (1/2) prepared extemporaneously is added and then the mixture is maintained at room temperature for 30 minutes until the spectrophotometer reading the absorbances at 750 nm against a blank prepared under the same conditions. A calibration curve was performed with bovine serum albumin (BSA) at differents concentrations (0-100 µg.mL⁻¹). Analyses were performed in triplicate.

NB:

- Solution A = Na₂CO₃ at 2% in NaOH 0,1 N
- Solution B₁ = CuSO₄ 5 H₂O at 1%
- Solution B₂ = C₄H₄KNaO₆ 4 H₂O at 2%
- Solution C = 250 µL of solution B₁ + 250 µL of solution B₂ + 24,5 mL of solution A prepared extemporaneously.

2.5.3 Analysis of caffeine, catechin and epicatechin by HPLC

The chromatographic analysis was performed on a liquid chromatographic system equipped with a Waters Alliance 2695 HPLC module (Milford, MA, USA) connected to a Waters 996 photodiode array (PDA) detector and column oven with a variable UV-vis detector. A quaternary pump, Waters 600 E, was used for high-pressure gradient elution. Data were collected, stored and analyzed using the EMPOWER software version 5.0 from Waters (Milford, MA, USA). Injections were made with an automatic injector Waters W717. The column used was a C18 Macherey-Nagel (250 mm x 4.6 mm – 5 µm Nucleodur - 100A °). 20 µL of sample are injected and the elution is carried out at a constant flow rate (0.8 ml / min) with a mobile binary phase consisted of A (95/5, H₂O/MeOH + 0.1% TFA) and B (100% ACN + 0.1% TFA). A gradient elution was performed by varying the proportion of solvent A to solvent B. The mobile phase composition started at 100% solvent A for 19 min, followed by a linear increase of solvent B to 6% for 31 min, 20% for 0.1 min and 100% for 9.9 min, and the bring mobile phase composition back to the initial conditions in 9.9 min for the next run. The washing cycle of the column is carried out after each injection. All the prepared solutions were filtered through 0.45 µm

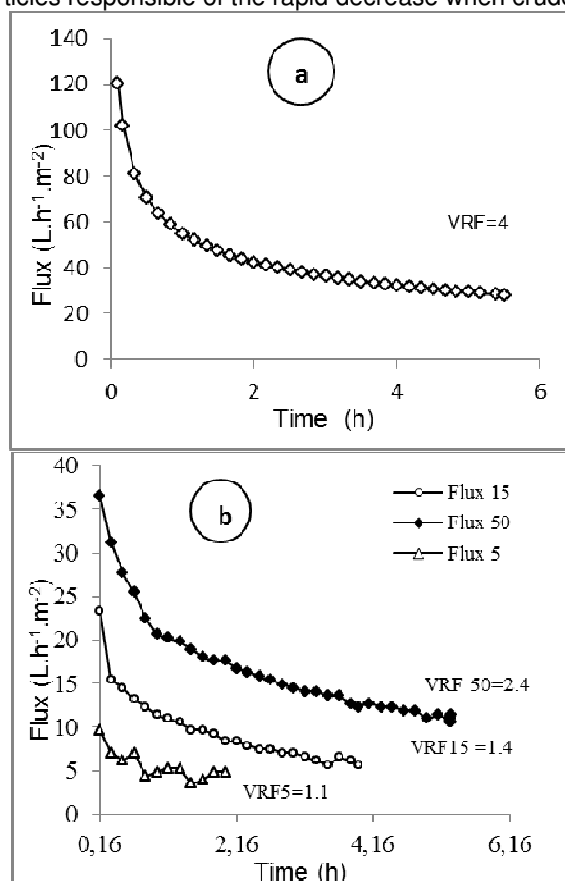
membranes (Fisher scientific). Absorption wavelength was selected at 274 nm. Chromatographic peaks in the samples were identified by comparing their retention time and UV spectrum with those of the reference standards. Working standard solutions (0 to 50 ppm) were injected into the HPLC, and peak area responses were obtained.

3. RESULTS AND DISCUSSION

3.1 Batch concentration on a pseudo-tangential pilot

Fig. 3 shows a curve of typical flux decline in time during pre-treated kola extract in microfiltration and ultrafiltration. The typical curves showed that the permeate flux decreased gradually with the operating times. This drop in flux is more pronounced during filtration of crude extract (Fig. 3a, 3b) and flows observed could be divided into three parts. A rapid initial drop (part I) followed by a long and term gradual flux decrease in part II and ended with a steady-state flux in the third part (III) [25,43]. For biological fluids such as kola extract flux decline in membrane filtration is a result of the increase of the membrane resistance due to particles which have low diffusion coefficient (macromolecules and colloids) [43]. This situation create an additional resistance in the flow through the membrane and can act as the second membrane which overlaps in the original membrane [44]. Balakrishnan *et al.* [45] demonstrated that the significant flux decline was due to the deposition of small particles and colloidal on the membrane surface which led to membrane fouling. According to Vladislavjevic *et al.* [46], the most problematic consequence of the membrane fouling when a membrane system is operated at constant pressure and cross flow velocity is a decrease, often accentuated sharp decline of the flow of permeation with time.

On the other hand, the permeate flux gradually decreases during the ultrafiltration of microfiltered extract (Fig. 3c). This permeate flux decrease would be due to elimination of a part of macromolecules and particles responsible of the rapid decrease when crude extract is used.



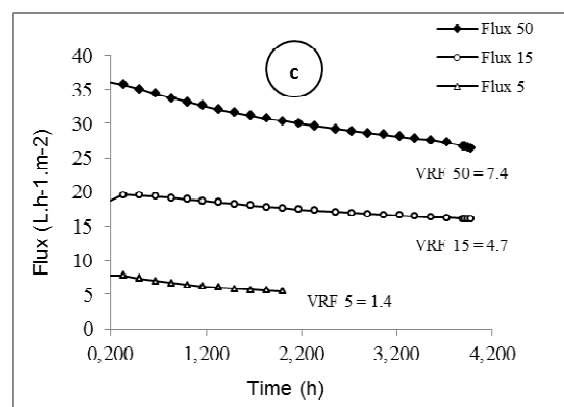


Fig.3: Effect of MWCO and the coupling step on pseudo-tangential pilot on relative flux of kola extract filtered through (a) MF 0.2 μm at TPM 0.6 bar, (b) UF without microfiltration, (c) UF with microfiltration at TPM= 2 bar. The crossflow velocity = 2.8 m/s and Temperature = 25°C are constant during operation.

The concentration polarization is therefore implemented gradually. Similarities flux decrease with MF and UF ceramic membranes have been observed in clarification of buckthorn berries juice with 0.2 μm ceramic membrane [35], orange juice with PVDF/PMMA 0.2 μm [47], artichoke wastewaters with 15 kDa ceramic membrane [16].

During ultrafiltration, the permeate fluxes are greater with the 50 kDa membrane than the 15 and 5 kDa. This is explained by the difference in the pore sizes of the membranes. In fact, during filtration through porous membrane, the permeability is proportional to the membrane pore size and the diameter of the molecules of the feed solution [48]. The permeability is higher; greater is the membrane pores size compared to the hydraulic resistance which is all the greater when the pore size is small [46-47]. This result in a greater volumetric reduction factor (VRF) for UF 50 kDa than 15 and 5 kDa. Similarities statements have been observed with UF ceramic membranes (15 and 50 kDa) in clarification of apple juice [49].

The flux values as well as the VRF remain nevertheless greater in UF after previous filtration by MF. During the filtration of the crude extract through UF membranes, the initial flux values are 36, 24 and 10 $\text{L.h}^{-1}\text{m}^{-2}$ compare to 10, 6 and 4 $\text{L.h}^{-1}\text{m}^{-2}$ at the steady-state for the UF 50, 15 and 5 kDa, respectively. This is equivalent to flux decreases of 73, 40 and 60%, respectively. The stabilized flux corresponds to 13, 8 and 5 $\text{L.h}^{-1}\text{m}^{-2}$ for UF 50, UF 15 and UF 5 kDa, respectively. Stabilization of this flow occurs much earlier with membranes of 5 and 15 kDa (60 and 150 min) compared to 260 min for UF 50. On the other hands, during the filtration of the MF permeate, the initial values are 36, 20 and 8 $\text{L.h}^{-1}\text{m}^{-2}$ compare to 27, 16 et 5 $\text{L.h}^{-1}\text{m}^{-2}$ at the end of the filtration for the UF 50, 15 and 5 kDa, respectively. Which corresponds to flux decrease of 25, 20, 37%, respectively. These various claims are confirmed by the retention rates of the compounds during concentration mode. During the filtration, molecules are more or less retained by the membranes (Table 2). In general, for each UF membrane, the retention rates of the compounds are lower during the filtration of the MF permeate. For each compound, the retention rate is lower with the UF 50 kDa. This would be justified by the fact that the permeation of the compounds is facilitated by the large molecular weight cut-off (MWCO) of the 50 kDa with respect to the 15 and 5 kDa. The clogging of the membrane increase rapidly with the membranes of 5 and 15 kDa but gradually with the 50 kDa. The permeability of the latter is therefore greater during the filtration period. The highly retained compounds at each filtration step are proteins and total phenols. This is explained by their high molecular weight, their affinity with the membranes and the ease of formation of aggregates [43]. According to Vernhet *et al.* [50] and Ulbricht *et al.* [19], total phenols react as acidic compounds (acceptor of electron pair or donor of H^{+}) due to their hydroxyl (OH) groups of phenolic nuclei and highlight the importance of the formation of H bonds in their physico-chemical reactivity. They also note that once the number of nuclei phenols is greater than two, the affinity of compounds to surfaces is greatly increased regardless of the polarity of the latter. Work carried out on the extraction and concentration of certain fruit juices also showed that the

proteins, by their high molecular weights, participated in the formation of a gel on the surface of the membranes and also in the blocking of the pores [51-53].

Table 2: Retention of compounds coming from pseudo-tangential filtration

	MF/UF5	MF/UF15	MF/UF50	UF5	UF15	UF50
Total phenols (%)	24.73	14.29	17.14	66.84	47.06	20.00
Proteins (%)	28.73	13.33	14.29	72.63	56.25	24.73
Caffeine (%)	9.31	5.00	0.31	16.05	2.34	1.28
Catechin (%)	15.7	5.67	2.03	21.46	8.70	2.54
Epicatechin (%)	12.97	7.53	2.24	24.98	12.48	2.74

MF/UF : Microfiltration coupled with ultrafiltration

Caffeine, catechin and epicatechin are poorly retained by the membranes. This indicates that their molecular weight is less than the membrane pores size. However, retention rates still remain high. In table 3 the mass balance of the MF and UF process for all compounds analyzed is reported. The general observation is that during the microfiltration or ultrafiltration of the crude extract of kola, the extraction yields are greater in the permeate MF. It can be noted that 66.06%, 67.56%, 46.21%, 70.98%, 71.44% and 70.77% of initial content of dry matter, total phenols, proteins, caffeine, catechin and epicatechin, respectively, were maintained in the permeate of the microfiltration process. Moreover, by comparing the quantities in each permeate UF during the filtration of this permeate, it is found that the mass recovery of the compounds is greater in the permeate of 50 kDa. More than 70% of the compounds are present in this permeate. This higher content would be due to the higher volumetric reduction factor (VRF) of 7.4 for UF50 compare to 4.7 and 1.4 for UF15 and UF5 kDa, respectively. Consequently, the coupling of steps (MF/UF) favors a greater covering of the molecules compared to the direct filtration on ultrafiltration.

Table 3: Mass balance (%) during batch concentration on pseudo-tangential pilot

	MF	MF/UF5	MF/UF15	MF/UF50	UF5	UF15	UF50
Dry matter (%)	66.06	7.25	78.17	86.21	5.91	20.57	49.85
Total phenols (%)	67.56	7.1	67.35	71.61	2.32	12.71	44
Proteins (%)	46.61	6.72	68.09	74.08	1.91	10.5	41.4
Caffeine (%)	70.98	8.55	74.64	86.16	5.87	23.44	54.29
Catechin (%)	71.44	7.95	74.11	84.68	5.5	21.91	53.6
Epicatechin (%)	70.77	8.21	72.66	84.5	5.25	21	53.49

The results presented are those of the balance sheet of the various permeates

3.2 Effect of operating parameters on tangential pilot filtration

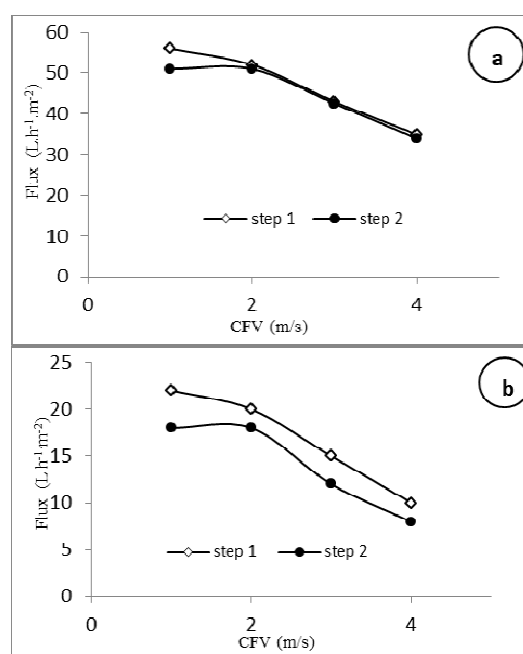
3.2.1 Effect of crossflow velocity on permeate flux

When tests were performed on recycle mode and various crossflow velocities (CFV) some unexpected results were produced. Fig.4 show data obtained for selected membrane under otherwise identical filtration conditions for each step. The permeate flux at constant pressure of 10 bar remains constant during lowest to highest CFV from 0.5 to 1.5 m/s in nanofiltration (Fig.4c). Similar statement is observed on the highest to lowest (from 1.5 to 0.5 m/s). The permeate flux is therefore independent of crossflow velocity applied. Similar flow stability observations were made in NF 70 fouling experiments with divalent cations (calcium) in natural organic matter [54]. In this study, permeate flux

remains constant when crossflow velocity increase from 4 to 40.4 cm/s. This could be explained by the fact that the membrane, due to the small diameter of its pores, is preferably permeable to the solution than to the solutes. As a result, only the applied pressure could influence the flow rate of the solvent through the membrane.

This phenomenon is slightly different from that observed in microfiltration and ultrafiltration (Fig.4 a et 4b). Indeed, during these two processes, the recorded permeate fluxes remain stable from 0 to 2 m/s and then decrease for the highest velocities. Similar statements are observed by Tarleton and Wakeman [53] during filtration of finer calcite suspensions through microfiltration (0.2 μm) membrane. According to Tarleton and Wakeman [55], when the challenge stream contained a greater proportion of larger, unground, particles the filtration rate was seen to fall with increasing crossflow velocity despite a substantial thinning of the fouling layer at the higher crossflows. A possible explanation for the phenomenon might be in terms of a particle classification near the filtering surface. It is known that the membrane membrane deposits which appear during microporous membranes are formed from the finer particle species present in the feed stream [56]. The axial velocity gradient which is generated across the flow channel would seem to cause a preferential deposition of the finer material from the feed at the membrane surfaces. This no entirely unknown mechanism (s) would preferential removed a larger particles from the foulant layers by the scouring action of the crossflow stream. The deposits responsible for fouling could thus have a resistance considerably higher than that which might be expected from a simplistic approach [53]. Moreover, during the decrease in the intensity of the velocity, the permeability of the membrane is not totally recovered during ultrafiltration compare to what is observed in microfiltration and nanofiltration. This could be explained by internal clogging of the UF membrane during the filtration of the permeate MF. Indeed, ultrafiltration is more prone to clogging by adsorption [48]. According to Azarte [48], El Rayess *et al.*[43], Cardoso de oliveira *et al.*[57], membrane fouling could be divided in function of its localization relative to the membrane structure : (i) internal fouling, caused by the adsorption and deposition of small particles and macromolecules within the internal structure of the pores, the irregularity of pore passages causes the particle to become tightly fixed blinding the pore (ii) external fouling, caused by the deposition of large macromolecules and particles on the top of the membrane. External fouling can be removed by relaxation of the considered parameter [25].

The optimum velocity determined for each membrane is 2 m/s for microfiltration and ultrafiltration, and 1 m/s for nanofiltration. This velocity makes it possible to obtain a large flux of permeate while minimizing the clogging of the membranes.



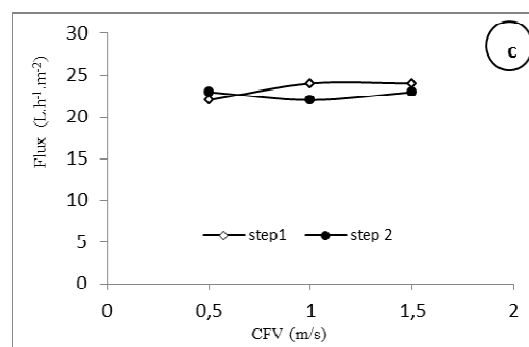
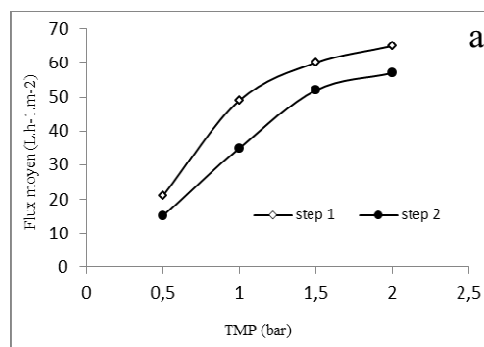


Fig.4. Effect of initial permeate flux on molecules fouling for various crossflow velocities. Results are presented flux vs crossflow: (a) microfiltration at 1bar, (b) ultrafiltration at 2 bar, (c) nanofiltration at 10 bar

3.2.2 Effect of transmembrane pressure on permeate flux

In order to observe the effect of pressure on the permeate flux, some experiments were carried out at constant crossflow velocity determined previously (Fig.5). In general, the results illustrate that the increase in applied pressure led to an increase in permeate flux values. According to Akdemir and Ozer [58], this statement is based on the Darcy's law. These data are in agreement with the findings of other authors [59,35]. It can be seen in microfiltration, when the pressure values up to 1.5 bar (Fig.5a), a constant value of flux is reached. This effect is caused by the formation of cake layer on the membrane surface, which accelerates the membrane fouling [58,25,60]. According to Aimar [61] and El-Rayess *et al.* [43], the permeate flux increases linearly with pressure until a maximum limit value or limit flux is reached. Beyond this value, a pressure difference is created on each side of the membrane which will lead to an increase in the concentration of solutes in the vicinity of the membrane. This over-concentrated polarization layer will oppose the transfer of the particles and thus reduce the permeate flux. In UF, this phase is reached from 2 bar, the flow tends to stabilize and thus becomes independent of the applied pressure. When the pressure is decreased (step 2), as seen during CFV effect, internal pores blocking of the membranes of MF and UF occurs, which results in flux values lower than the initial values. The observation is a slightly different in NF. Indeed, a stability of the permeate flux is noted during highest to lowest pressure (Fig. 5c). This phenomenon could be explained by the compaction or conditionnement of the NF membrane before reducing transmembrane pressure (step 2). According to Azarte [48], A preconditioning of the membrane under a precise pressure of the maximum pressure is often recommended to obtain reproducible performances in terms of permeability.

The optimum pressure of each membrane is thus determined, which is the applied pressure value allowing a high permeate flux to be obtained while inducing a low clogging of the membranes. This pressure value is 1, 3 and 10 bar for the MF, the UF and the NF, respectively.



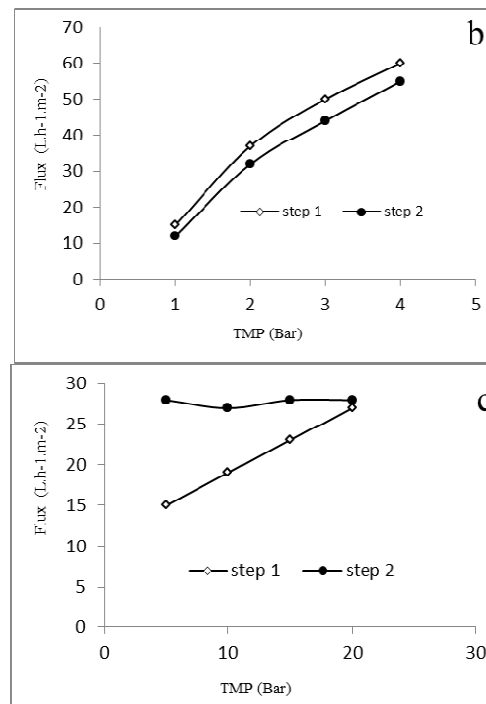


Fig.5. Effect of initial permeate flux on molecules fouling for various pressures. Results are presented flux vs pression: (a) microfiltration at 2 m/s, (b) ultrafiltration at 2 m/s, (c) nanofiltration at 1 m/s.

3.3 Batch concentration mode on tangential pilot filtration

By applying the optimum filtration conditions previously determined, the MF, UF and NF membranes were used for the clarification-purification-concentration of a crude cola extract. In general, as seen previously, results showed that the permeate flux in microfiltration and ultrafiltration decreased with the operating times by increasing of the volumetric reduction factor (VRF) due to polarization and gel formation (Fig 6a, 6b). The initial permeate flux of about 133 and 52 L.h⁻¹.m⁻² reached a steady-state value of about 36 and 33 L.h⁻¹.m⁻² when the VRF was 4.4 and 6.8, for MF and UF, respectively. Thus, after filtration and rinsing of the reversible fraction with deionized water, membranes performances determined (Table 4) reveal the lower permeability coefficients L_{p1} of 132.8 and 12.53 compare to initial value L_{p0} which are 261.2, 67.33 L.h⁻¹.m⁻².bar⁻¹. This induces an additional resistance of these membranes (irreversible resistance R_{irrev}): 1.1×10^{12} and 2.03×10^{13} m⁻¹ for microfiltration and ultrafiltration, respectively. This translates a membrane permeability loss of 43 and 80%. Kola extract therefore contains molecules capable of reducing the permeabilities of the membranes during the microfiltration and ultrafiltration.

Table 4 : Membranes performances during batch concentration

	Permeability L_{p0} (L/h m ² .bar)	Permeability L_{p1} (L/h m ² .bar)	Permeability loss (%)	R_{irrev} (m ⁻¹)	Recovery rate%
MF 0.2 μm	261.2	132.8	43	1.1×10^{12}	99
UF 50 kDa	67.33	12.53	80	2.03×10^{13}	96
NF*	1.14	1.14	0	0	nd
NF**		0.4	65	6.2×10^{14}	

Mean values ± standard deviation of duplicate determinations

$$\mu_{EtOH50\%} = 9,82 \cdot 10^{-4}; \text{ Temperature: } 25^{\circ}\text{C}$$

$$L_{p0} = \text{membrane initial permeability}$$

$$L_{p1} = \text{permeability after filtration of the extract and rising}$$

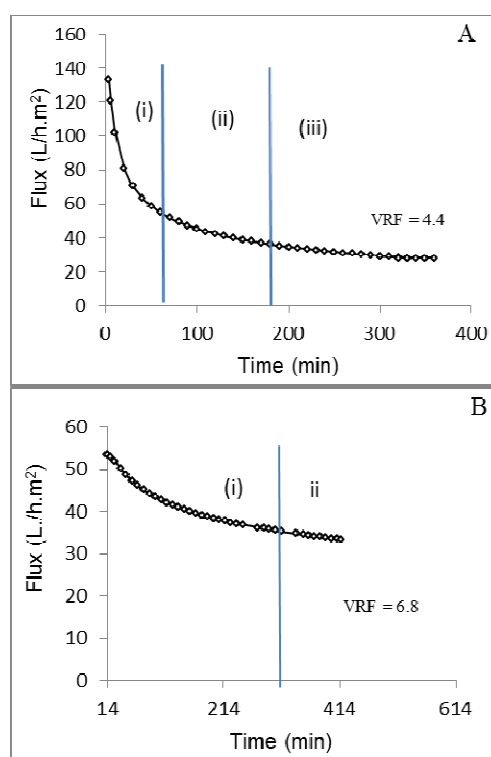
* : filtration of UF permeate (MF/UF/NF)
 ** : filtration of crude extract

The clarified kola extract by coupling MF/UF was concentrated by nanofiltration in optimal operating conditions (Fig. 6c). It showed the evolution of the permeate flux according to batch concentration mode in which, starting from 2.05 L of clarified extract, 1.52 L of permeate were produced (final VRF, 3.8). The initial permeate flux of $20 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$ decreased rapidly in the first 17 min; then permeate flux reached a steady-state permeate value of about $17 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$ until to the final VRF, ie a flow loss of 15%. This decrease in flux could be attributed to the polarization concentration and fouling of membrane. This behavior was similar to that observed by Diaz-Reinoso *et al.* [26] and Murakami *et al.* [31] in the nanofiltration process of (1st) aqueous extracts from distilled fermented grape pomace when using pressure between 2 and 4 bar (2nd) aqueous mate from *Ilex paraguariensis* at 3 bar.

This gradually decrease could be explained by the concentration polarization and subsequent cake layer formation (Cassano *et al.*, 2015). Thus, the coefficient of permeability observed after rinzing the membrane ($L_{p1} = 0.4 \text{ L/h m}^2 \text{ bar}$) was lower than initial value ($L_{p0} = 1.14 \text{ L/h m}^2 \text{ bar}$). This indicates a $R_{irrev} = 6.2 \times 10^{14} \text{ m}^{-1}$, 100 times greater than that obtained during microfiltration. This being due to the smaller pore size in NF and the operating conditions applied for each membrane. According to Pagliero *et al.* [47] permeability is higher; greater is the membrane pores size, compared to the hydraulic resistance which is all the greater when the pore size is small.

It can be seen that microfiltration and ultrafiltration steps therefore influence the performance of the NF membrane. According to Paraskeva *et al.* [63] the pretreatment of the extract by ultrafiltration would be a prerequisite for concentration by nanofiltration due to the elimination of colloids and particles responsible of membrane fouling. Differences observed in permeate fluxes and VRFs show that the direct concentration of the prefiltered kola extract could be an alternative to step coupling. It remains to be seen which of the procedures would favor a high recovery of the different compounds of interest.

The recovery rates determined are between 96 and 99%, which reflects the effectiveness of the washing method used.



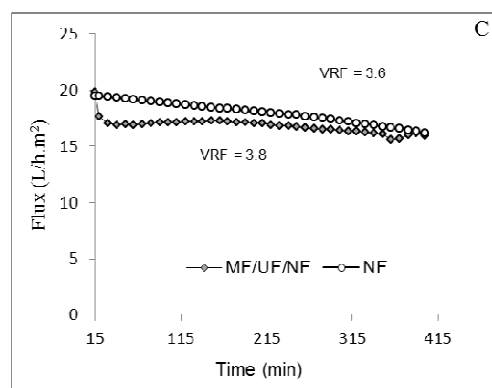


Fig. 6 : Schematic representation of kola extract filtration at 25°C (A) microfiltration, TMP =1 bar, CFV=2 m/s, flow = 150 l/h), (B) ultrafiltration, TMP =3 bar, CFV= 2 m/s, flow = 150 l/h), (C and D) nanofiltration, TMP =10 bar, FV=1 m/s, flow =120 l/h).

3.7 Impact of microfiltration and ultrafiltration on kola extract quality

There is a general decrease in the content of the compounds analyzed when passing from the feed stream to the permeate (Table 5). This indicates compounds retention by the various membranes tested. In microfiltration, the rejection coefficients are 35.71, 6.82, 2.35, 2.10 and 1.46% for proteins, total phenols, epicatechin, caffeine and catechin, respectively. According to El-Rayess *et al.* [43], the high retention of proteins and other suspended particles promotes clarification of the extract tested. Consequently, a purity gain of the other interest compounds transmitted in the permeate is observed. The microfiltration jointly promotes the retention of the macromolecules and a gain in purity of the total phenols and caffeine. During the ultrafiltration, similar results are observed. However, nearly 30% of polyphenols are retained by the membrane. Which results in a decrease in the purity of the latter in the final permeate.

3.8 Concentration by nanofiltration

Table 6 shows the different extraction yields of the compounds during MF/UF/NF coupling and direct concentration of crude extract through NF. In general, there is a decrease in the content of the compounds analyzed when passing from the feed stream to the permeate. This indicates compounds retention by the various membranes tested. The highly retained compounds at each experiment step are proteins and total phenols with retention index (R) of 100 when VRF was 3.6 and 3.8 for direct concentration by NF and coupling MF/UF/NF, respectively. These compounds are responsible of the membrane fouling observed previously. This indicates that their molecular weight is greater than the pore size of the NF 270 membrane used. This result was better than those observed by Murakami *et al.* [31] and Bras *et al.* [39] in the concentration of phenolic compounds of aqueous propolis extract and *Cynara cardunculus* through nanofiltration, achieving an R of 84% and near 100 %, respectively, of phenolic compounds.

In the other hands, as seen in table 6, it is possible to see that of all the catechin, epicatechin and caffeine, present in the initial solution (UF permeate), only 13.89%, 16.07% and 27.42%, respectively, were lost to the permeate. These data are for the most part less than those observed during the direct concentration of the crude extract. There are, in fact, 6.83%, 9.26% and 18.76% for catechin, epicatechin and caffeine, respectively. Thus, the passage of these compounds through the membrane is due to their low molecular mass which is near the the cut-off membrane (200 – 400 Da). Differences in retention rates are the result of a different chemical composition of the feed solutions. Despite the lost of compounds, the value obtain represent a high retention index and corroborate that the NF process is suitable for the concentration of water-ethanol mixture extract of kola nut.

During the concentration of the ultrafiltration permeate, 56.73%, 54.13%, 28.31%, 65.46%, 57.99% and 72.13% of initial content of dry matter, total phenols, proteins, caffeine, catechin and epicatechin, respectively, were maintained in the final retentate of the process. These extraction yields are, however, lower than those obtained during the direct concentration of the feed stream (76 - 99%). This implies that a large fraction of these compounds is retained in the prior steps of microfiltration and ultrafiltration, as seen before. The concentration factors observed during nanofiltration (Table 5) indicate higher concentrations of the molecules of low molecular masses that are caffeine (4.13), catechin (3.66) and epicatechin (4.55) during the coupling mode compared to those obtained by direct concentration of crude extract. While the inverse is observed for total phenols and proteins. This could be explained by the fact that the retention of the macromolecules and colloids during the MF/UF steps would have favored the purification of the kola extract. Thus, at identical VRF and dry matter, reducing the feed volume promotes a higher concentration of the purified extract compounds relative to the crude extract. Previous studies for the concentration of compounds present in aqueous or water alcohol mixture plant extract through nanofiltration, using a membrane with a MWCO of 150-300 Da [31],[64] achieved a concentration factor (CF) of 5 with a VRF of 4. This CF was higher than that obtained in our study. According to authors, modifications in the equipment's operational parameters such as pressure, temperature, duration of the process, membrane type and pore size, raw material, and VRF cause different behaviors in a process of separation through membranes [31],[39],[21].

Table 5: Retention coefficient and purity of compounds during microfiltration and ultrafiltration

	Crude extract	MF permeate	%R	MF retentate	Purity	UF permeate	%R	UF retentate	Purity
Dry matter (g/L)	2.25	2.05	8.89	2.68	-	1.96	4,54	2,49	-
Total phenols (g/L)	1.17	1.09	6.82	1.41	1	0.77	29,17	1,49	0.7
Proteins (g/L)	0.03	0.02	35.71	0.08	0.7	0.01	35,29	0,05	0.6
Caffeine (mg/L)	84.83	83.05	2.10	90.07	1	80.45	3,13	99,66	1
Catechin (mg/L)	69.9	68.88	1.46	74.84	1	67.80	1,56	84,18	1
Epicatechin (mg/L)	13.59	13.27	2.35	14.62	1	12.76	3,84	17	1

*Filtration conditions: Microfiltration (MF): TPM = 1 bar, CFV = 2 m/s, T= 25 °C
Ultrafiltration (UF): TPM = 3 bar, CFV = 2 m/s, T= 25 °C
%R: rejection coefficient*

Table 6 : Content of filtered kola extract

	Coupling MF/UF/NF					Direct Nanofiltration (NF)			
	Feed	Permeate	Retentate	Balance mass (%)	FC	Permeate	Retentate	Balance mass (%)	FC
Dry matter (g/L)	2.25	0	8.00	56.37	nd	0	8	87.64	nd
Total phenols (g/L)	1.17	0	4.00	54.13	2.56	0	5	99.71	4.27
Proteins (g/L)	0.03	0	0.05	28.31	1.79	0	0.12	93.33	4
Caffeine (mg/L)	84.83	22.06	350.24	65.46	4.13	15.92	277.66	76.37	3.27
Catechin (mg/L)	69.9	9.42	255.7	57.99	3.66	4.77	259.19	86.52	3.7
Epicatechin (mg/L)	13.59	2.25	61.83	72.13	4.55	1.25	48.11	82.60	3.54

*Concentration conditions : CVF= 1 m/s, TPM = 10 bar, T=25 °C, Q=120 L/h
FC= concentration factor
nd= not determined*

4. CONCLUSION

The objective of this study was the study of membrane potential in the context of the extraction, purification and concentration of a hydro-alcoholic extract of kola. It can be told that the kola nut extract can be concentrated by membrane separation. The operating conditions defined for each stage allowed the purification - concentration of the kola extract and better performances of these membranes. Kola extract filtration through microfiltration and ultrafiltration membranes was perfect; the fouling of the membrane was rapid in MF and gradually in UF. A large amount of proteins was retained in these two steps. This retention promotes a gain of purity of compound of interest such as caffeine, catechin and epicatechin. On the other hand, the high retention of the polyphenols during the ultrafiltration, impacts on the extraction yield of these compounds during the final concentration. However, direct concentration of the extract has more advantageous data on extraction yields and operating time. But, membrane clogging is significant and requires subsequent physical or chemical treatment before any reuse. Thus, the steps of microfiltration and ultrafiltration contribute to the purification of the feed stream but significantly reduce the content of the compounds, especially the polyphenols during ultrafiltration. However, the high extraction yield observed for each compound at the end of the concentration justifies the industrial interest that could be the extract of kola. The process could easily be adapted for other extract types. Taking into account the transmission of the compounds by the NF membrane, further studies should be done to separate extracts into different fractions of interest more or less important for the different industries.

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