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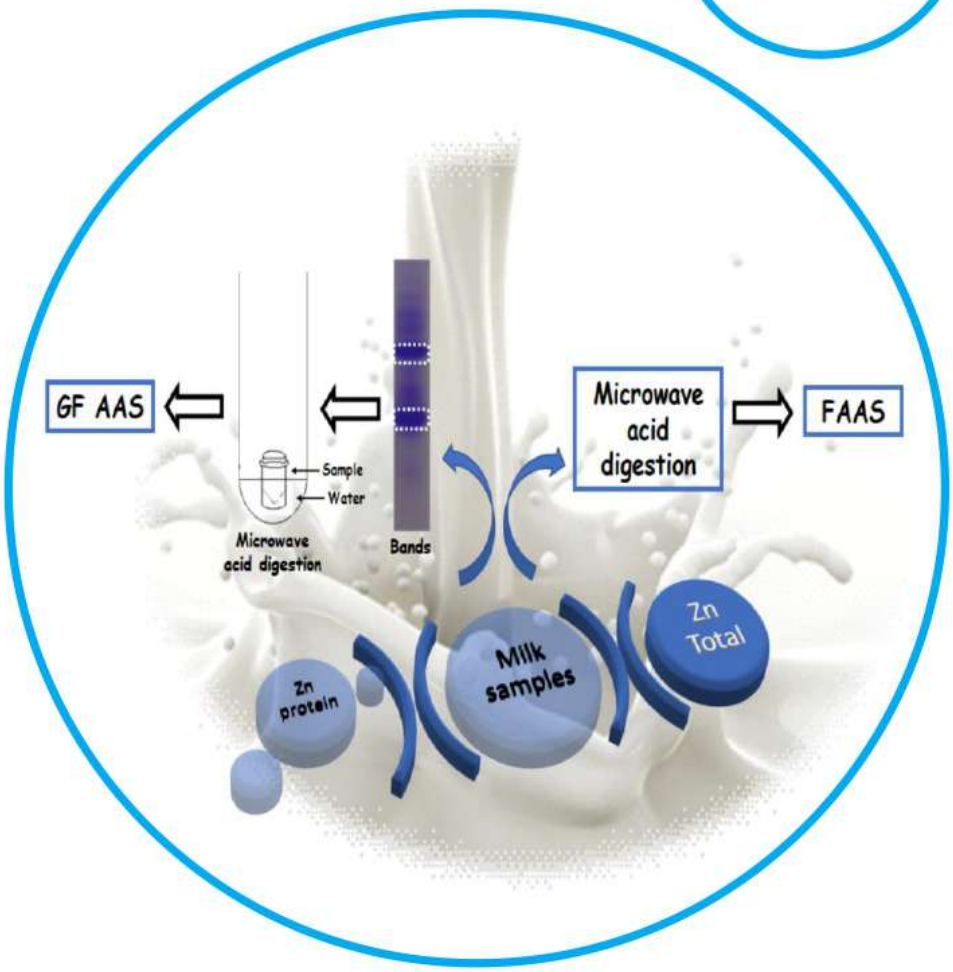
Zinc fractionation in cow, goat, sheep and soybean milk samples using gel-electrophoresis and determination by electrothermal atomic absorption spectrometry (ETAAS)



Impact of salinity on the kinetics of CO_2 fixation by *Spirulina platensis* cultivated in semi-continuous photobioreactors

Analytical procedures for the determination of estrogen compounds in a surface water reservoir in Southeast Brazil

Chemical profiles and antimicrobial activities of plants utilized in Brazilian traditional medicine

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Editorial

Wishing all readers a prosperous new year, the Editor of Eclética Química Journal, Editorial Board and Staff proudly present the first edition of 2021, with the certainty that EQJ authors and readers will feel rewarded with the published findings. This first issue of 2021 begins with the description of a screening method for zinc levels in milk samples from raw cow, raw sheep, UHT cow, UHT goat and soybean-based milk to establish the Zn levels' differences in protein samples. The total Zn levels in the extracts were determined by flame absorption atomic spectrometry. After protein fractionation and separation by urea polyacrylamide gel electrophoresis, zinc-protein analysis was performed by electrothermal atomic absorption spectrometry, indicating that the method provided a quantitative and accuracy determination of Zn species present in the protein fractions of the milk samples. Next article deals with the investigation of the physiological response of the microalgae *Spirulina platensis* to salinity stress. *Spirulina platensis* and *Spirulina platensis* adapted to high salt concentration were operated at laboratory scale in a semi-continuous photobioreactors to analyze the rate of carbon dioxide biofixation. The authors concluded that the kinetic parameters estimated for *Spirulina platensis* can be used to improve photobioreactor design for reducing of atmospheric carbon dioxide. In the sequence, results on the antimicrobial activity against several fungi and bacteria are presented for five medicinal plants popularly indicated for treating common infectious diseases in Recife, Brazil. This study was motivated because in Brazil there are hundreds of street markets selling a wide variety of herbs for medicinal purposes without quality control or scientific evidence; instead, their purported efficacy is based exclusively on empirical ethnobotanical knowledge. The results confirmed that the five traditional medicinal plants are efficient low-cost alternative sources of extracts to treat infections, especially for people living in abject poverty. The following article describes the validation of an analytical methodology to determine and quantify four estrogen hormones present in water using high-performance liquid chromatography with detections by diode array detector and by fluorescence detector. Five surface water sampling campaigns were carried out in five different sites of Furnas Reservoir over three months and the presence of estrogen hormones was detected. Closing this issue, two polyethersulfone hollow fiber membranes were fabricated containing 16 and 20% of polyethersulfone with N-methyl-2-pyrrolidone as solvent and tap water as nonsolvent. The effect of the polymer content on the properties of the membranes was evaluated analyzing their morphological properties, response to tensile tests and pure water permeability. The results showed that the only relevant statistical difference was pure water permeability, being ~25% better in the 16% polymer membrane, implying a minor energy consumption to produce the same volume of permeate.

In this opportunity the Editor and members of Editorial Board of Eclética Química Journal present the sincerest recognition to the effort made by authors and Reviewers during the last year and hope to continue with this essential collaboration in 2021.

Assis Vicente Benedetti
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[2] Wendlant, W. W., Thermal Analysis, Wiley-Interscience, New York, 3rd ed., 1986, ch1.

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SUMMARY

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Zinc fractionation in cow, goat, sheep and soybean milk samples using gel-electrophoresis and determination by electrothermal atomic absorption spectrometry (ETAAS)

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ABSTRACT: A screening method for zinc levels in different milk samples (raw cow, raw sheep, UHT cow, UHT goat and soybean milk base) was performed to establish the Zn levels' differences in protein samples. The samples were digested in a cavity microwave oven and the total Zn levels in the extracts were determined by flame absorption atomic spectrometry (FAAS). The protein separation was performed by urea polyacrylamide gel electrophoresis (UREA-PAGE). Protein bands were digested in the cavity microwave oven and Zn-protein analysis was further conducted by electrothermal atomic absorption spectrometry (ETAAS). The results showed that Zn is mainly bound to 32 kDa (β-casein) protein (17.0 ± 2.0%) in UHT cow and 24 kDa (α-casein) protein (9.0 ± 0.6%) in raw sheep milk. This method provided quantitative information regarding Zn species present in the protein fractions of the milk samples. The accuracy was evaluated using certified reference material (whole milk powder, NIST 8435) with statistically equivalent concentrations (Student's *t*-test) for total Zn and by addition and recovery experiments applied to measure Zn-protein. The recovered values were in the 92-110% range.

1. Introduction

Zinc (Zn) is a trace element essential in human and animal nutrition and participates in critical biological processes, such as the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids. It is present in food in two forms: bound with organic molecules and in inorganic salts[1]. Milk is one of the main sources of zinc in the human diet, responsible for about 25% of the total recommended daily intake of this metal[1,2]. Milk is a complete source of nutrients, containing minerals, vitamins, carbohydrates, lipids, water and proteins, which are especially crucial for newborns' growth and development[3].

As humans continue to consume milk during childhood and adulthood, highlighting the expressive consumption of cow's milk, it is essential to understand better the chemical composition of milk, including the mineral levels and their physiological influence on human and animal nutrition, as well as trace elements toxicity[2].

Studies about different chromatographic methods have indicated differences in the association between Zn and proteins in human and cow's milk. In cow's milk, the complex Zn-casein-Ca-P is the predominant form, while in human milk, Zn-citrate species seem to be predominant[4]. In the environmental, biological, medical and biochemical research areas, studies about metal-protein interaction are necessary to improve the knowledge about the toxicity, bioavailability, transport and physicochemical properties of an element[5].

Nutrient fractionation in food is necessary to understand availability and absorption prediction. Fractionation is defined as the classification process of an analyte or an analyte group in a specific sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties[6]. One relatively simple procedure to fractionate proteins from food samples is the polyacrylamide gel electrophoresis, which shows high selectivity and consists in the separation of molecules loaded in a particular way under the influence of an electric potential difference[7]. One of the main uses of the gel electrophoresis technique containing urea is the characterization of proteins in various types of milk[8].

Studies about metal-protein interaction using polyacrylamide gel electrophoresis, followed by different analytical techniques for metals determination, have been developed for several kinds of application[9-16]. These works belong to an important field of science called metallomic, which has allowed for the integration of analytical studies with inorganic and biochemical studies. Therefore, there is a growing demand for accurate and selective procedures that allow the quantification and speciation of trace elements, especially in foods, to obtain relevant information about metal-protein binding, which is intimately related to the nutrients bioavailability[7,17].

In this context, the present work aimed at investigating the separation of proteins in different kinds of milk samples, by urea polyacrylamide gel electrophoresis (UREA-PAGE), and to establish Zn(II) concentrations in each protein determined by electrothermal atomic absorption spectrometry (ETAAS). The Zn distribution results in protein bands were quantitative and provided information about Zn levels present in the milk samples' protein fractions.

2. Experimental

2.1 Instrumentation

A Varian model SpectrAA-800 graphite furnace atomic absorption spectrometer (Mulgrave, Victoria, Australia) equipped with a graphite furnace atomizer, GTA 100 autosampler, was used for zinc quantification in the proteins bands. Background correction by Zeeman-effect was employed to correct for non-specific absorbance. Pyrocoated graphite tubes (Part Number 63-100011-00, Varian) furnace with longitudinal heating was throughout. All signals were measured as integrated absorbance. A zinc hollow cathode lamp (λ = 213.9 nm, slit = 1.0 nm) operating in 5 mA was employed as radiation source. Argon 99.998% (Air liquid Sao Paulo, Brazil) was used as purge gas. The heating programs used after the optimization of pyrolysis and atomization temperatures are shown in Tab. 1.

The total Zn was carried out using a flame atomic absorption spectrometer (model SpectrAA 250 plus, Varian, Mulgrave, Australia) equipped with a Zn hollow cathode lamp (λ = 213.9 nm, slit = 1.0 nm) and 5 mA lamp current were employed as primary radiation sources. Air/acetylene flame was used at 13.4 L min^{-1} and 2 L min^{-1} air and acetylene flows, respectively.

The Mini-gel (Vertical Electrophoresis System Mini, BioAmerica Inc. Equipments, Miami, USA) with 8.5 cm (height) x 10.0 cm (width) and a centrifuge (5417R, Eppendorf, Hamburg, Germany) were used for gel-electrophoresis.

The milk samples were microwave digested assisted (Multiwave, Anton Paar, Graz, Austria) with 50 mL PFA vessels. The total protein amount was determined by spectrophotometry (Spectrophotometer model 432, Femto, Sao Paulo, Brazil).

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Table 1. GF AAS heating program used in the zinc protein bands.

Step	Temperature / ºC	Hold Time / s	Ar Flow / L min^{-1}	Reading
Drying	85	2.0	3.0	No
Drying	95	8.0	3.0	No
Drying	120	10	3.0	No
Pyrolysis	1000	5.0	3.0	No
Pyrolysis	1000	1.5	3.0	No
Atomization	1600	0.8	0	Yes
Atomization	1600	2.9	0	Yes
Cleaning	2500	2.9	3.0	No

2.2 Reagents and materials

All solutions were prepared using deionized water (resistivity > 18.2 MΩ cm) obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Nitric acid (HNO_3) (Synth, Diadema, SP, Brazil) previously purified using a sub-boiling distillation system (Milestone, Sorisole, Italy), and hydrogen peroxide (H_2O_2) 30 % w v^{-1} (Sigma Aldrich, Switzerland) were used to digest the samples.

A solution of 1.78% (m/v) $Mg(NO_3)_2$ (Merck, Darmstadt, Germany) was used as a chemical modifier. Analytical reference solutions between 0.2 and 1.0 mg L^{-1} of Zn were prepared by successive dilutions of a stock solution containing 1000 mg L^{-1} Zn (Tec-Lab, Hexis, Jundiai, SP, Brazil).

The analytical curve for Zn determination in protein bands was obtained using analytical solutions with concentrations between 0.5 and 3.0 µg L^{-1} Zn in blank gel band previously microwave-assisted digested with HNO_3 1.0% (v/v).

2.3 Samples

Fresh milk samples from Holstein cows aged between 3 to 4 years, weighing an average of 450 kg and raised in a semi-intensive system, and sheep from Santa Inês were collected at the Embrapa Pecuária Sudeste, located in São Carlos, SP, Brazil. Samples of ultra-high temperature (UHT) cow milk, UHT goat milk and soybean milk base were purchased in the local market of São Carlos, SP, Brazil. Samples were stored in a fridge at – 4 ºC before analysis.

Certified reference milk sample (NIST 8435 - Whole Milk Powder) from the National Institute of Science and Technology (NIST, Gaithersburg, MD, USA) was used to check the methods' accuracy for the measurement of total Zn. Addition and recovery experiments were performed to evaluate the species of Zn.

2.4 Procedures

2.4.1 Evaluation of pyrolysis and atomization temperature

The pyrolysis and atomization temperature curves were constructed with 18 µL of the standard or sample solutions in the presence of a chemical modifier, 5 µL of $Mg(NO_3)_2$ 1.78% (w/v). The temperatures were evaluated in a range of 700-1800 °C using increments of 100 ºC. Solutions of 1.0 µg L^{-1} Zn were prepared for this evaluation using the blank sample, a piece of polyacrylamide gel without protein band, in acid digested (HNO_3 1.0% v/v).

2.4.2 Sample preparation for total Zn and species determination

Zinc's total mass fractions were determined in the milk samples using a microwave-assisted acid digestion. In brief, 2.5 mL of milk samples or 250 mg of the certified reference material was directly weighted in the microwave vessel. Then, 1.0 mL of H_2O_2 30% (w/v), 1 mL of H_2O and 1.0 mL of HNO_3 65% (v/v) were added and the mixture submitted to the following microwave heating program: 5 min from 0 to 100 W, 5 min at 600 W, 5 min at 1000 W followed by 15 min of cooling. After digestion and cooling, the digests were transferred to volumetric flasks and the volume was made up to 20 mL with deionized water.

Protein bands (8 mm wide by 3 to 5 mm in height) of the milk samples and the blank (gel region without the protein) obtained by electrophoresis were cut, washed with deionized water and dried on filter paper for 15 min in the laminar flow cabinet before weighting (each band was approximately 50 mg). The bands were placed in PTFE mini-bottles, and 75 µL 65% (v/v) HNO_3 plus 75 µL 30% (w/v) H_2O_2 were added.

The mini bottles were placed into the microwave PFA vials containing 2 mL deionized water. Figure 1

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shows the container configuration used. The microwave heating program was run as follows: 1 min from 0 to 250 W, 1 min at 0 W, 5 min at 250 W, 5 min at 400 W, 10 min at 750 W followed by 10 min of cooling. After digestion and cooling, the digests were transferred to volumetric flasks and the volume was made up to 3.5 mL with deionized water.

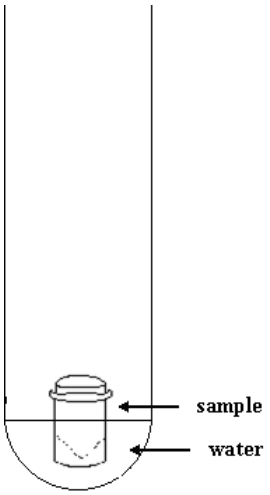

Figure 1. Sample container into the vial of the microwave cavity vessel.

2.4.3 Gel Electrophoresis

Urea-polyacrylamide gel electrophoresis (UREA-PAGE) was carried out using a separation gel composed of acrylamide (4% for stacking gel and 10% for separation gel), as described by Egito *et al.*[18], with modifications.

The separation gel was composed of 2.25 mL acrylamide/Bis (40 g acrylamide (Invitrogen, USA) and 2 g N,N-methylene bisacrylamide (BIS) (Sigma, Germany) in 100 mL solution) and 7.10 mL separation gel buffer (6.43 g of TRIS (Synth, SP, Brazil), 38.57 g urea (J.T. Baker, Germany), 0.572 mL HCl (Aldrich Chemical, Milwaukee, WI, USA), with pH adjusted to 8.9 in 100 mL solution).

The stacking gel was prepared using 0.39 mL acrylamide/Bis and 3.52 mL stacking gel buffer (0.830 g TRIS, 30 g urea, 0.44 mL HCl, with pH adjusted to 7.6 in 100 mL solution). Both gels also contained N,N,N',N'-tetramethylethylenediamine (TEMED) (J.T. Baker, Germany) and 10% (m/v) ammonium persulfate (Sigma, Germany). Finally, Tris-glycine buffer solution (3.75 g TRIS and 18.25 g glycine (Sigma, Germany) in 250 mL of solution, diluted 5 times) was used in the reservoir.

To sample preparation, 30 µL of samples and standard curve of purified protein markers (GE Healthcare) were dissolved in 1.0 mL of buffer solution, composed by 0.375 g TRIS, 24.5 g urea, 0.2 mL HCl, 0.35 mL β-mercaptoethanol (Inlab, Brazil) and 0.075 g bromophenol blue (Merck) diluted to 50 mL with water. After dissolution, the samples were immersed in a water bath at 40 °C for 1 h and centrifuged at 5 °C for 10 min at 10000×g. Then, 30 µL of supernatant was applied to a single slot. The initial and final currents were 23 and 24 mA and the voltage was set at 90 V for 4.5 h. After the protein migration, protein bands were stained overnight with a solution containing 0.5 g of Coomassie brilliant blue G-250 (Sigma, Germany) and 3.75 mL of H_2SO_4 (Quemis, Brazil) in 500 mL solution. This solution was then filtered using filter paper and mixed to 55.6 mL of 10 mol L^{-1} KOH and 66.67 mL of 12% (w/v) trichloroacetic acid (Synth, Brazil). Finally, the gels were washed with deionized water and stored in 10% (v/v) glycerol 10% (v/v) methanol solution (Proquímios, Brazil).

The molecular weight proteins were estimated according to standard purified protein markers, including phosphorylase b (97.0 kDa), albumin (66.0 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (30.0 kDa), trypsin inhibitor (20.1 kDa) and α–Lactalbumin (14.4 kDa). The gel electrophoresis UREA-PAGE was performed in triplicate for each sample and separately to avoid contamination.

2.4.4 Determination of Total Protein concentration

The total protein concentration was determined through the Bradford method[19] by adding 1.25 mL of the Bradford reagent (Coomassie Brilliant Blue G-250) and 100 µL of bovine serum albumin (BSA) (Sigma, USA) standard solutions or the samples and measuring the absorbance after 5 min at 595 nm, in triplicate. The analytical curve was constructed by using standard solutions of BSA at concentrations between 50 and 250 mg L^{-1}, prepared by successive dilutions of a stock solution containing 1000 mg L^{-1} of BSA.

3. Results and discussion

3.1 Gel Electrophoresis

Figure 2 shows the gels electrophoresis obtained for each milk sample, where columns 2 to 6 correspond to the protein bands of each sample, and column 1 shows

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the low molecular weight (LMW) standard protein (14.4 to 97 kDa). The dashed rectangles represent the bands studied with the approximate molecular weight, as this is a qualitative analysis. The protein molecular mass values, found from top to bottom, were 52 and 39 kDa for the soybean milk base (column 2), 32 and 24 kDa for raw sheep (column 3), 32 and 24 kDa for UHT cow (column 4), 32 kDa for UHT goat (column 5) and 32 and 24 kDa raw cow (column 6).

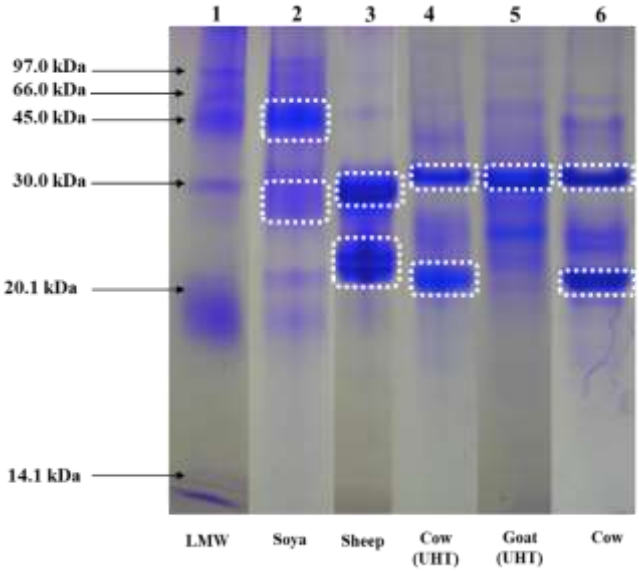

Figure 2. Zinc protein bands of milk samples run in polyacrylamide gel (10%) with urea.

Casein is the major component of milk protein. In this work, α-casein, β-casein and soybean proteins showed profiles similar to other studies[18,20,21] with approximately 24 kDa and 32 kDa for α-casein and β-casein, respectively. Bands of higher intensity for raw sheep, UHT cow, raw cow and UHT goat were in the molar mass range of 32 kDa. For the soybean milk base, the bands 52 and 39 kDa were assigned to β-conglycine subunits and glycine, respectively[20,22].

Naqvi *et al*.[23] reported that it was also possible to observe that Ca, Fe, Mg and Zn were mainly associated with colloidal calcium phosphate in casein micelles. For soybeans, 80% of the extracted proteins are β-conglycine (7S) and glycine (11S). Denaturation by heating occurs when there are interactions between these proteins, which can form basic subunits, such as (11S), β-subunits (7S), and α-,α'-subunits (7S)[20,22].

3.2 Total Zn and species determination

Among the micronutrients present in milk, Zn has the highest concentration and is important in human and animal nutrition, associated with organic molecules and present as inorganic salts. Concerning amino acids such as histidine and methionine and phosphates and organic acids, it enables the bioavailability of this element[1]. It is also associated with one of the most important milk proteins, casein, and in the bovine milk, Zn is present as Zn-casein-Ca-P complex[4].

The total zinc levels obtained in the samples are shown in Tab. 2 and agree with those reported in the literature[24,25]. No significant difference (Student's *t*-test 95% level, p>0.05) was found between the certified reference material NIST 8435 (28 ± 3 mg kg^{-1}) and the measured value of certified reference material (26 ± 1 mg kg^{-1}) for Zn. Therefore, this study can confirm the accuracy of the procedure. The slope and correlation coefficient (r) of the FAAS calibration curve prepared in the presence of 1.0% (v/v) of HNO_3 were 0.4688 and 0.9994, respectively. Moreover, limits of detection (LOD) calculated as three times the standard deviation of the blank (n=10)/slope was 0.02 mg L^{-1} and quantification (LOQ) (3 × LOD) was 0.03 mg L^{-1} for Zn, which are appropriate for Zn determination in the evaluated milk samples[26].

The pyrolysis and atomization temperature were evaluated to find the Zn measurement's best conditions in proteins by ETAAS (Fig. 3). The drying and cleaning temperatures were used according to Bossu *et al*.[27]. Pyrolysis temperature was set at 1000 °C and the atomization temperature was set at 1600 ºC. In this condition, the absorbance analytical signals' best profiles and the smallest deviations between the measurements were observed. The coefficient of variation was around 3.0% (n = 3) and the graphite furnace heating program used to Zn determination in the protein bands of different milk samples is shown in Tab. 1.

The slope and correlation coefficient (r) of ETAAS analytical curve in the presence of blank sample media (gel without protein) were 0.1457 and 0.995, respectively. The method's accuracy was evaluated by the addition and recovery procedure. Recoveries of 110 ± 1%, 92 ± 3%, 99 ± 4%, 95 ± 8% were obtained after adding 1.5 µg L^{-1} of Zn to proteins bands 32 kDa UHT cow, 32 kDa UHT goat, 39 kDa soybean and 24 kDa raw sheep, respectively. These recovery values are in the range established according to the quantified concentration level[28,29]. LOD and LOQ for Zn determination by ETAAS, using the optimized conditions, were 0.20 µg L^{-1} and 0.66 µg L^{-1}, respectively.

The results for Zn distribution in protein bands were quantitative and provide information about the relative

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Zn concentration in each protein band from milk samples, as well as its relationship with the total levels of Zn found in milk samples (Tab. 2). Zinc is present predominantly in 32 kDa protein in UHT cow milk, followed by 32 kDa raw cow milk and 24 kDa raw sheep milk, which showed similar Zn levels.

Table 2. Zinc mass fractions (%) obtained for protein bands (Zn-protein) by ETAAS and Total Zinc (mg L^{-1}) obtained for milk samples (ZnT) by FAAS, n = 3.

Concentration/% ± standard uncertainty[a], Concentration/mg L^{-1} ± standard uncertainty[b] and coefficient of variation (CV) / %				
Samples / kDa	Zn-protein / %[a]	CV / %	Total Zinc / mg L^{-1} [b]	CV / %
Soybean milk-based / 52	n.d	n.d	2.50 ± 0.07	2.8
Soybean milk-based / 39	4.4 ± 0.6	12.6		
UHT Cow / 32	17.0 ± 2.0	9.4	3.20 ± 0.02	0.6
UHT Cow / 24	8.0 ± 1.0	17.6		
UHT Goat / 32	5.2 ± 0.9	16.3	2.75 ± 0.01	0.4
Cow / 32	8.0 ± 1.0	8.3	3.44 ± 0.05	1.5
Cow / 24	n.d	n.d		
Sheep / 32	1.7 ± 0.2	9.7	2.76 ± 0.01	0.4
Sheep / 24	9.0 ± 0.6	6.8		

n.d = not detected.

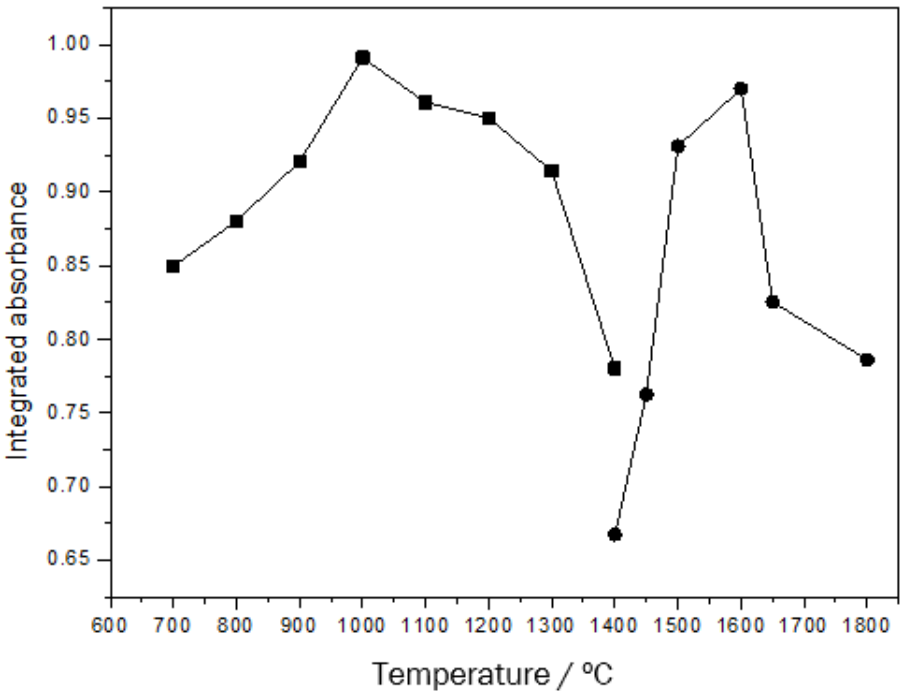

Figure 3. Pyrolysis (■) and atomization (●) temperature curves for 1.0 µg L^{-1} Zn in the blank (absence of band gel) in 1.0% v/v HNO_3 with 1.78% w/v $Mg(NO_3)_2$ of chemical modifiers.

It can be concluded that Zn is present in these samples in metal-binding proteins. There were no differences between the Zn-binding proteins' estimated values in UHT cow milk (8.0 ± 1.0%) and raw cow milk (8.0 ± 1.0%), which presented the highest Zn amount in 24 and 32 kDa, respectively. However, the largest proportion of Zn found in the 32 kDa protein was in UHT cow milk (17.0 ± 2.0%) and 24 kDa protein (9.0 ± 0.6%) in raw sheep milk. Studies were conducted to determine Zn bound to casein in breast milk, in which processed milk samples presented a change in the Zn distribution, with a decrease in the serum fraction and an increase in the fat fraction. There was no significant difference in the Zn percentage regarding casein. However, there was a trend towards increasing in processed samples[30], as observed in this study.

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Previous studies[9,19,31,32] showed that zinc could also be linked to the casein fraction, α-casein and β-casein in bovine milk. The complex fractions of α-k-casein and α-β-casein showed significant zinc bound amounts, as can be demonstrated in this work.

Gaucheron *et al*.[31] estimated that cations are bound to α-casein and β-casein in the following affinity order: Fe > Zn > Ca. Zinc was found with 3 CPP (casein phosphopeptide) and the fraction containing α-casein and the 3 CPPs containing amino acids (glutamic acid, serine and phosphoserine), showing the Zn-complexes formation[23,32]. Moreover, the Zn distribution between casein, whey and other components may be affected by pH, heat and other cations present in the diet. Thus, the proteins are denatured and their ability to bind to Zn is probably reduced[33-35]. This reduction can be explained by the Zn percentage found in some proteins such as soybean (52 kDa), raw cow (24 kDa) and raw sheep (32 kDa).

Although contamination or species conversion problems cannot be ignored, gel electrophoresis is an important tool for the fractionation or separation of compounds, such as caseins, and Zn distribution can provide valuable information about the proteins' activity and their associated components.

3.3 Determination of total protein

Lipid extraction is not necessary during the Bradford method, so total protein determination was made directly in the diluted milk[36]. The aim was to observe if the ultra-high temperature (UHT) process caused changes in the milk total protein concentration when compared with the unprocessed samples. Results obtained for raw sheep, raw cow, UHT cow, UHT goat and soybean were 34 ± 1 g L^{-1}, 21 ± 1 g L^{-1}, 23 ± 1 g L^{-1}, 18 ± 1 g L^{-1} and 16 ± 1 g L^{-1}, respectively. No significant difference was observed in the results that compared processed and not processed milk, in agreement with a previous work[30].

Soybean is a protein supplier food composed by saturated and unsaturated fatty acids, vitamins and polyphenolic compounds, such as isoflavones[35]. It may be prepared to have the same protein content as bovine milk[34,35], which can be confirmed in this work (16 ± 1 g L^{-1}). However, the biological value of soybean proteins is lower than bovine milk or eggs proteins[35].

Proteins found in goat milk showed results comparable to bovine milk. The highest total protein concentration was found in sheep's milk, in agreement with the results reported by Raynal-Ljutovaca *et al*.[24] and was similar to bovine milk.

4. Conclusions

This study evaluated a fractionation method for Zn-proteins measurement in different milk samples by ETAAS. A method for total Zn quantification in different milk samples was also studied.

The Zn distribution results in protein bands were quantitative and could provide information about the relative Zn concentration in milk proteins. It was also possible to define the most appropriate polyacrylamide gels preparation procedure, the milk sample preparation and Zn determination. No differences were observed between the estimated values of Zn-binding proteins in UHT cow milk and in raw cow milk, which presented the highest Zn concentrations in 24 and 32 kDa, respectively. Total protein content in processed (UHT) and non-processed (raw) milk samples were also remarkably similar. The results showed that Zn is mainly bound to 32 kDa (β-casein) protein in UHT cow and 24 kDa (α-casein) protein in raw sheep milk. The use of fractionation has been demonstrated as a complementary analytical tool for the characterization of Zn species present in milk.

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References

[1] Scheplyagina, L. A., Impact of the mother's zinc deficiency on the women's and newborn's health status, Journal of Trace Elements in Medicine and Biology 19 (1) (2005) 29-35. https://doi.org/10.1016/j.jtemb.2005.07.008.

[2] Ballard, O., Morrow, A. L. Human Milk Composition: Nutrients and Bioactive Factors, Pediatric Clinics of North America 60 (1) (2013) 49-74. https://doi.org/10.1016/j.pcl.2012.10.002.

[3] Hodgkinson, A. J., Wallace, O. A. M., Smolenski, G., Prosser, C. G., Gastric digestion of cow and goat milk: Peptides derived from simulated conditions of infant digestion, Food Chemistry 276 (2019) 619-625. https://doi.org/10.1016/j.foodchem.2018.10.065.

[4] Michalke, B., Element speciation definitions, analytical methodology, and some examples, Ecotoxicology and Environmental Safety 56 (1) (2003) 122-139. https://doi.org/10.1016/S0147-6513(03)00056-3.

[5] Arruda, M. A. Z., Azevedo, R. A., Metallomics and chemical speciation: towards a better understanding of metal-induced stress in plants, Annals of Applied Biology 155 (2009) 301-307. https://doi.org/10.1111/j.1744-7348.2009.00371.x.

[6] Templeton, D. M., Ariese, F., Cornelis, R., Danielsson, L.-G., Muntau, H., van Leeuwen, H. P. V., Lobinski, R., Guidelines for terms related to chemical speciation and fractionation of elements. Definitions, structural aspects and methodological approaches (IUPAC Recommendations 2000), Pure and Applied Chemistry 72 (8) (2000) 1453-1470. https://doi.org/10.1351/pac200072081453.

[7] Shi, W., Chance, M. R., Metalloproteomics: forward and reverse approaches in metalloprotein structural and functional characterization, Current Opinion in Chemical Biology 15 (1) (2011) 144-148. https://doi.org/10.1016/j.cbpa.2010.11.004.

[8] Dupont, D., Croguennec, T., Pochet, S., Milk Proteins – Analytical Methods, Reference Module in Food Science (2018) 1-14. https://doi.org/10.1016/B978-0-08-100596-5.22616-4.

[9] Pozzi, C. M. C., Braga, C. P., Vieira, J. C. S., Cavecci, B., Queiroz, J. V., Barbosa, H. S., Arruda, M. A. Z., Gozzo, F. C., Padilha, P. M., Metal ions bound to the human milk immunoglobulin A: Metalloproteomic approach, Food Chemistry 166 (2015) 492-497. https://doi.org/10.1016/j.foodchem.2014.06.040.

[10] Aslebagh, R., Channaveerappa, D., Arcaro, K. F., Darie, C. C., Comparative two dimensional polyacrylamide gel electrophoresis (2D-PAGE) of human milk to identify dysregulated proteins in breast cancer, Electrophoresis 39 (14) (2018) 1723-1734. https://doi.org/10.1002/elps.201800025.

[11] Vacchina, V., Oguey, S., Ionescu, C., Bravo, D., Lobinski, R., Characterization of metal glycinate complexes by electrospray Q-TOF-MS/MS and their determination by capillary electrophoresis–ICP-MS: application to premix samples, Analytical and Bioanalytical Chemistry 398 (2010) 435-449. https://doi.org/10.1007/s00216-010-3907-1.

[12] Santos, F. A., Lima, P. M., Neves, R. C. F., Moraes, P. M., Pérez, C. A., Silva, M. O. A., Arruda, M. A. Z., Castro, G. R., Padilha, P. M., Metallomic study on plasma samples from Nile tilapia using SR-XRF and GFAAS after separation by 2D PAGE: initial results, Microchimica Acta 173 (2011) 43-49. https://doi.org/10.1007/s00604-010-0522-y.

[13] Neves, R. C. F., Lima, P. M., Baldassini, W. A., Santos, F. A., Moraes, P. M., Castro, G. R., Padilha P. M., Fracionamento de cobre em proteínas do plasma, músculo e fígado de tilápia do Nilo. Química Nova 35 (3) (2012) 493-498. https://doi.org/10.1590/S0100-40422012000300010.

[14] Wang, Q., Bernhard Michalke (Ed.): Metallomics: analytical techniques and speciation methods, Analytical and Bioanalytical Chemistry 409 (2017) 5617–5618. https://doi.org/10.1007/s00216-017-0524-2.

[15] Magalhães, C. S., Arruda, M. A. Z., Sample preparation for metalloprotein analysis: A case study using horse chestnuts, Talanta 71 (5) (2007) 1958-1963. https://doi.org/10.1016/j.talanta.2006.08.039.

[16] Wang, Q., Xiong, Y. L., Zinc-binding behavior of hemp protein hydrolysates: Soluble versus insoluble zinc-peptide complexes, Journal of Functional Foods 49 (2018) 105-112. https://doi.org/10.1016/j.jff.2018.08.019.

[17] Silva, M. S., Sele, V., Sloth, J. J., Araujo, P., Amlund, H., Speciation of zinc in fish feed by size exclusion chromatography coupled to inductively coupled plasma mass spectrometry using fractional factorial design for method optimisation and mild extraction conditions, Journal of Chromatography B 1104 (2019) 262-268, https://doi.org/10.1016/j.jchromb.2018.11.010.

[18] Egito, A. S., Rosinha, G. M. S., Laguna, L. E., Miclo, L., Girardet, J. M., Gaillard, J. L., Método eletroforético rápido para detecção da adulteração do leite caprino com leite bovino, Arquivo Brasileiro de Medicina Veterinária e Zootecnia 58 (5) (2006) 932-939. https://doi.org/10.1590/S0102-09352006000500032.

[19] Gomez, B. G., Perez-Corona, M. T., Madrid, Y., Availability of zinc from infant formula by in vitro methods (solubility and dialyzability) and size-exclusion chromatography coupled to inductively coupled plasma-mass spectrometry, Journal of Dairy Science 99 (12) (2016) 9405-9414. https://doi.org/10.3168/jds.2016-11405.

[20] Liu, W., Lou, H., Ritzoulis, C., Chen, X., Shen, P., Lu, Y., Wu, K., Dong, L., Zhu, H., Han, J., Structural characterization of soybean milk particles during *in vitro* digestive/non-digestive simulation, LWT 108 (2019) 326-331. https://doi.org/10.1016/j.lwt.2019.03.086.

[21] Maqsood, S., Al-Dowaila, A., Mudgil, P., Kamal, H., Jobe, B., Hassan, H. M., Comparative characterization of protein and lipid fractions from camel and cow milk, their functionality, antioxidant and antihypertensive properties upon simulated gastro-intestinal digestion, Food Chemistry 279 (2019) 328-338. https://doi.org/10.1016/j.foodchem.2018.12.011.

[22] Ren, C., Tang, L., Zhang, M., Guo, S., Interactions between whey soybean protein (WSP) and beta-conglycinin (7S) during the formation of protein particles at elevated temperatures, Food Hydrocolloids 23 (3) (2009) 936-941. https://doi.org/10.1016/j.foodhyd.2008.06.007.

[23] Naqvi, M. A., Irani, K. A., Katanishooshtari, M., Rousseau D., Disorder in Milk Proteins: Formation,

Structure, Function, Isolation and Applications of Casein Phosphopeptides, Current Protein & Peptide Science 17 (4) (2016) 368-379. https://doi.org/10.2174/1389203717666151201191658.

[24] Raynal-Ljutovac, K., Lagriffoul, G., Paccard, P., Guillet, I., Chilliard, Y., Composition of goat and sheep milk products: An update, Small Ruminant Research 79 (1) (2008) 57-72. https://doi.org/10.1016/j.smallrumres.2008.07.009.

[25] Güler, Z., Levels of 24 minerals in local goat milk, its strained yoghurt and salted yoghurt (tuzlu yoğurt), Small Ruminant Research 71 (1-3) (2007) 130. https://doi.org/10.1016/j.smallrumres.2006.05.011.

[26] Pereira Junior, J. B., Fernandes, K. G., Müller, R. C. S., Nóbrega, J. A., Palheta D. C., Determinação direta de Ca, Mg, Mn e Zn em amostras de leite de búfala da ilha de marajó por espectrometria de absorção atômica com chama (FAAS) Química Nova 32 (9) (2009) 2333-2335. https://doi.org/10.1590/S0100-40422009000900018.

[27] Bossu, C. M., Carioni, V. M. O., Naozuka, J., Oliveira, P. V., Nomura, C. S., Direct determination of arsenobetaine and total As in robalo fish liver and tuna fish candidate reference material by slurry sampling graphite furnace atomic absorption spectrometry (SLS-GF AAS), Eclética Química Journal 44 (2) (2019) 37-44. https://doi.org/10.26850/1678-4618eqj.v44.2.2019.p37-44.

[28] Taverniers, I., Loose, M., Van Bockstaele, E., Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance, TrAC Trends in Analytical Chemistry 23 (8) (2004) 535-552. https://doi.org/10.1016/j.trac.2004.04.001.

[29] Chudzinka, M., Debska, A., Baralkiewicz, D., Method validation for determination of 13 elements in honey samples by ICP-MS, Accreditation and Quality Assurance 17 (2012) 65-73, https://doi.org/10.1007/s00769-011-0812-z.

[30] Góes, H. C. A., Torrese, A. G., Donangelo, C. M., Trugo, N. M. F., Nutrient composition of banked human milk in Brazil and influence of processing on zinc distribution in milk fractions, Nutrition 18 (7-8) (2002) 590-594. https://doi.org/10.1016/S0899-9007(02)00813-4.

[31] Gaucheron, F., The minerals of milk, Reproduction Nutrition Development 45 (4) (2005) 473-483. https://doi.org/10.1051/rnd:2005030.

[32] Miquel, E., Farré, R., Effects and future trends of casein phosphopeptides on zinc bioavailability, Trends in Food Science & Technology 18 (3) (2007) 139-143. https://doi.org/10.1016/j.tifs.2006.11.004.

[33] Pabón, M. L., Lönnerdal, B., Bioavailability of zinc and its binding to casein in milks and formulas, Journal of Trace Elements in Medicine and Biology 14 (3) (2000) 146-153. https://doi.org/10.1016/S0946-672X(00)80003-6.

[34] Jung, S., Murphy, P. A., Sala I., Isoflavone profiles of soymilk as affected by high-pressure treatments of soymilk and soybeans, Food Chemistry 111 (3) (2008) 592-598. https://doi.org/10.1016/j.foodchem.2008.04.025.

[35] Zaheer, K., Akhtar, M. H. An updated review of dietary isoflavones: Nutrition, processing, bioavailability and impacts on human health. Journal Critical Reviews in Food Science and Nutrition 57 (6) (2017) 1280-1293. https://doi.org/10.1080/10408398.2014.989958.

[36] Kamizake, N. K. K., Gonçalves, M. M., Zaia, C. T. B. V., Zaia, D. A. M., Determination of total proteins in cow milk powder samples: a comparative study between the Kjeldahl method and spectrophotometric methods, Journal of Food Composition and Analysis 16 (4) (2003) 507-516. https://doi.org/10.1016/S0889-1575(03)00004-8.

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Impact of salinity on the kinetics of CO_2 fixation by *Spirulina platensis* cultivated in semi-continuous photobioreactors

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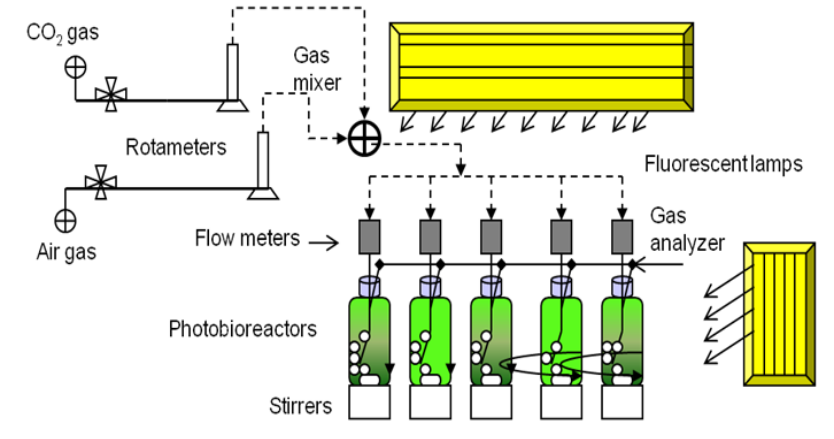
ABSTRACT: In this research, the physiological response of the microalgae *Spirulina platensis* to salinity stress (1 and 100 g L^{-1}) was investigated. *Spirulina platensis* and *S. platensis* (adapted to high salt concentration) were operated at laboratory scale in a semi-continuous photobioreactors. The responses examined were within 0.5 to 10% CO_2 concentration, temperatures from 10 to 40 °C, light intensities from 60 to 200 µmol m^{-2} s^{-1} and presented better results in terms of all kinetic parameters. The highest rate of CO_2 biofixation for *S. platensis* was 25.1 g_{CO2} m^{-3} h^{-1}, and the maximum specific growth (μ_{max}) achieved was 0.44 d^{-1} - 0.67 d^{-1} at 2.5% CO_2, 150 µmol m^{-2} s^{-1} at 25 °C. Corresponding determined values of *S. platensis* adapted were 18.2 g_{CO2} m^{-3} h^{-1}, 0.31 d^{-1} - 0.58 d^{-1} at 2.5% CO_2, 60 µmol s^{-1} m^{-2} and 28 °C. However, both microalgae exhibited experimental limiting growth factors, CO_2 10%, 40 °C and 200 µmol m^{-2} s^{-1}, conditions under which photosynthetic CO_2 biofixation may be inhibited and photoinhibition of photosynthesis may be enhanced by salinity. The efficiency of 2.5% CO_2 removal by *S. platensis* achieved 99%, whereas *S. platensis* adapted to 96%, respectively. The kinetic parameters estimated for *S. platensis* can be used to improve photobioreactor design for reducing of atmospheric carbon dioxide.



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1. Introduction

Global warming is generally attributed to greenhouse gases (GHG) increase in the atmosphere, particularly carbon dioxide (CO_2), for which atmospheric concentration has already achieved 387 ppm and needs to get down to 350 ppm or less in order to avoid global climate change consequences[1,2]. By 2100, 26 billion tons of CO_2 are estimated to be released into the atmosphere from anthropogenic sources[3]. Photosynthetic organisms such as microalgae species are potent producers of value-added bioactive compounds such as pigments, vitamins and long-chain polyunsaturated fatty acids, when grown under stress conditions can accumulate significant quantities of total lipids[4-6]. Recent studies indicated that improvements in culture conditions are needed to obtain adequate productivity of lipid, protein, carbohydrate content. It is well known that numerous parameters influence the growth of these compound content in microalgae: CO_2 addition, light, temperature, salinity, nutrient addition, inoculation size, stirring, pH, etc.[4,7]. The National Aeronautics and Space Administration (NASA) was the first institution to become interested in microalgae *Spriulina* for oxygen production, CO_2 reduction and proposed it as one of the primary foods to be cultivated in a future bioregenerative life support system for long-term manned space missions' scenarios such as Moon and Mars bases[4-6]. The cyanobacterium *Spirulina platensis* (*S. platensis*) is commercially produced as a nutrient source in health food, feed and pharmaceutical industries, especially in developing countries[8]. *S. platensis* has shown ability of adaptation to quite different habitats and colonizes harsh environments, where life is exceedingly difficult for other organisms. For example, in the lakes containing salt concentrations > 30 g L^{-1}, the cyanobacterial population became practically monospecific and *Spirulina* was the only organism present in significant quantities. Indeed *S. platensis* was found in waters containing from 20 to 270 g L^{-1} of salt, but growth seemed to be optimal at salt concentrations ranging from 20 to 70 g L^{-1} and it is possible that the population of *S. platensis* found at the highest salt concentrations, such as in temporary ponds just before drying, was that of the cyanobacterial biomass established when the concentration of salts was much lower[7]. *S. platensis* are thermophilic algae with optimal growth temperature between 35 to 37 °C[8]. When *S. platensis* was cultivated outdoor under high natural sunlight and salinity-stress, its production was usually accompanied by photosynthesis photoinhibition. Furthermore, it was suggested that salinity-stress enhances photosynthesis photoinhibition in green alga *Chlamydomobas reinhardtii*[9]. Thus, *S. platensis* incorporates into a suitable photo-bioreactor configuration that can enhance photosynthesis by increasing growth conditions and controlling exposure of *S. platensis* to environmental factors, as well as suitable for greenhouse gases attenuation, particularly converting CO_2 into biomass in which carbon is biofixed and incorporated into carbohydrates, lipids and proteins[10,11]. The microalgae biomass produced can also be used for various applications, such as biofertilizer, soil conditioner, and biofuels production[8,12]. However, more research and development are necessary on strain of microalgae selection, acclimation, and adaptation with regards to salt tolerance and the impact of other environmental parameters. In this paper, were studied the impacts of light intensity, temperature, and inlet CO_2 concentration on the specific growth rate of *S. platensis* and *S. platensis* adapted to salinity-stress during biofixation of CO_2 in photobioreactors at laboratory scale.

2. Experimental

2.1 Algal strain and cultivation conditions

Spirulina platensis from the American Type Culture Collection (ATCC) strain 53844 was cultivated in Zarrouk's culture[10] fresh medium described in Tab. 1, adjusted (autoclave medium) to a final pH 9.0 ± 0.2. The stock culture was maintained in a 250 mL Erlenmeyer flask containing 50 mL of the medium at 20 °C under 60 µmol m^{-2} s^{-1} of light intensity, and 16/8 h day/night cycle. Every week the culture was transferred to a 500 mL flask containing the respective fresh medium and acclimatized to 0.5% of CO_2 mixed with air. Then, for further tests, the acclimated culture was transferred into each photobioreactor and the CO_2 concentration was increased gradually by bubbling CO_2 (2.5%, 5%, 7.5% and 10%) for 24 h before starting the test at a flowrate of 0.05 L m^{-1}.

Adaptation of *S. platensis* to high salinity, an inoculum of microalgae *S. platensis* were cultivated in a modified Zarrouk's medium[10] (Tab. 2).

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Table 1. Composition of stock solutions used to prepare nutrient solution for *S. platensis**.

Stock Solution	Composition	Stock solution / g L^{-1}
Nutrient Solution	$NaHCO_3$	16.8
	K_2HPO_4	0.5
	$NaNO_3$	2.5
	K_2SO_4	1
	NaCl	1
	$MgSO_4.7H_2O$	0.2
	$CaCl^2{\cdot}2H_2O$	0.04
	$FeSO_4.7H_2O$	0.01
	EDTA	0.08
Trace metals mix A5	H_3BO_3	2.86
	$MnCl_2{\cdot}4H_2O$	1.81
	$ZnSO_4{\cdot}7H_2O$	0.222
	$NaMoO_4.2H_2O$	0.39
	$CuSO_4{\cdot}5H_2O$	0.079
	$Co(NO_3)^2{\cdot}6H_2O$	0.0494
Trace metals mix B6 modified	NH_4NO_3	0.23
	$K_2Cr_2(SO_4)_4{\cdot}24H_2O$	0.096
	$NiSO_4{\cdot}7H_2O$	0.0478
	$Na_2WO_4{\cdot}2H_2O$	0.0179
	$Ti(SO_4)_3$	0.040

*Preparation: Combine salt solution ingredients with 1 mL trace metals A5 mix and 1 mL trace metals B6 to prepare 1 L, adjust medium for final pH 9.0, and autoclave at 121 °C for 15 min. Adapted from "Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors", by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 67.

Table 2. Composition of stock solutions used to prepare nutrient salt solution for *S. platensis* (adapted).

Stock Solution	Composition	Stock solution / g L^{-1}
Salt nutrient solution	NaCl	100.0
	Seawater (Aquarium salt)	16.0
	$NaNO_3$	0.51
	Na_2SO_4	1.23
	$MgCl_2$	0.033
Trace metal mix† Vitamin solution†	$EDTA.2H_2O$	4.36
	$CoCl_2.6H_2O$	0.010
	$FeCl_3.6H_2O$	3.15
	$MnCl_2.4H_2O$	0.018
	$CuSO_4.5H_2O$	0.010
	$Na_2MoO_4.2H_2O$	0.0063
	$ZnSO_4.7H_2O$	0.022
	$NaH_2PO_4.2H_2O$	4.0
	Thiamine-HCl	2.0
TV solution*	Biotin	0.005
	Vitamin B_{12}	0.005

Preparation: Trace Metal mix solution†: Dissolve EDTA first in hot water and then combine with the other ingredients to 1L. Vitamin solution†: After combining the ingredients adjust to pH 6 filter sterilized (Do not autoclave). TV solution*, prepare the ingredients in 100 mL. Filter sterilized (Do not autoclave). Adapted from "Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors", by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 68.

This adapted culture followed the same experimental procedure for *S. platensis* as explained above. Experiments were designed in parallel photobioreactors and operated under the same experimental conditions to study the adaptation process of *S. platensis* to salinity.

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2.2 Photobioreactors and experiments

Figure 1 shows the graphical abstract scheme of the experimental set up at laboratory scale. The input CO_2 gas concentration was controlled by mixing CO_2 and air directly to each photobioreactor of 2 L working volume (WV) glass (Pyrex). The input gas mixture was connected by PVC tube (d=0.15 cm). The flowrate of CO_2 was measured by a flowmeter (Colepalmer) and a sintered sparge (porous air diffuser) placed into the photobioreactor for bubbling the into the biomass. The exhaust gas from each photobioreactor was connected by a PVC tube of 0.15 cm diameter and the flowrate measured by a flowmeter. Each photobioreactor was inoculated with 200 mL of precultured *S. platensis* and filled with 1800 mL nutrient solution prepared and mixed 24 h in advance to reach about 0.5 g L^{-1} concentration of suspension biomass. An experimental factorial design was proposed to study the three factors: CO_2 concentration, temperature and light intensity, ranging at five levels using the same experimental procedure, first for *S. platensis* culture followed by *S. platensis* adapted to high salt concentration. Five parallel photobioreactors were set up in the same chamber, the initial concentration of the cell biomass was approximately 0.5 g L^{-1}, the photobioreactors operated at the same temperature and light intensity, but the cell biomass in each photobioreactor received different CO_2 concentrations (0.5, 2.5, 5.0, 7.5 and 10%) under continuous bubbling of CO_2 at a rate of 0.5 L m^{-1}, and all run lasted 12 days. The following experiments were examined at varied temperatures of 15, 20, 25, 30 and 40 °C and then light intensities of 60, 80, 100, 150 and 200 µmol m^{-2} s^{-1}.

The photobioreactors temperature was maintained constant by immersing the photobioreactors in acrylic open water baths (0.46 x 0.25 x 0.8 m) with immersion circulator analog controller (Isotemp 2100). The light intensity was generated by cool white fluorescent tubes (General Electric 40w to 80w) and measured using a quantum sensor (model LI-190SA), connected to a quantum/radiometer/photometer light meter (model LI-250A, Li Cor Inc. Lincon, NE, USA). The quantum sensor was configured to make measurements of photon flux density (PDF) in the PAR (Photosynthetic Active Radiation, 400-700 nm). The source of CO_2 was provided by Airgas Specialty Gases New Jersey, 200 and 300 cubic feet volume cylinders of CO_2 concentrations of 0.5, 2.5, 5.0, 7.5 and 10% balance with air, the concentrations certified by the vendor.

Figure 1. Experimental setup of algal bioreactors at small scale.
Adapted from "Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors", by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 67.

2.3 Analytical determinations

The concentration of microalgae was measured by the method of filtration, being 10 mL suspension of cell biomass filtered on membrane filter (GF/C filters 1.2 µm, d=47 mm). After dried filters at 80 °C for 24 h, cell biomass weights were determined until achieved constant weight over time. The ratio of carbon in the dry cell biomass [Cc] was determined by ignition, dried cell biomass was ignited at 500 °C in a Thermolyne Furnace (model 62700, Thermolyne Corp. Dubuque, IA) to estimate the dry weight biomass. The cell biomass concentration for both microalgae was also determined as the changes in optical density (OD). The OD of the suspension algal biomass was measured at 680 nm (*S. platensis*) as absorbance, using a spectrophotometer (UV-VIS Shimadzu- 1700). The cell dry weight of *S. platensis* and optical density (OD_{680}) were established by linear regression (dry cell biomass, g L^{-1} = 0.477 × OD_{680} + 0.376; R^2 = 0.957; p = 0.01). Likewise, for *S. platensis* adapted and cell biomass g L^{-1}=2.35 × OD_{680} + 0.32; R^2=0.94, p=0.01. Triplicate samples of the cell biomass were collected every day until reached maximum microbial growth (4-5 g L^{-1}), depending upon the experimental conditions some photobioreactors were shut down earlier than others. The pH of the suspension of cell biomass was measured with a pH meter (Acumet ABIS Plus) calibrated with standard pH solutions of 4, 7 and 11. Analysis of CO_2 input/output of each bioreactor was measured with a Gas Chromatograph Shimadzu 17A

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with TDC detector, gas samples were taken in plastic bags. The CO_2 concentration was also monitored analyzing CO_2 directly in the gas stream off the bioreactor using colorimetric gas detection tubes RAE systems.

2.4 Determination of growth rate and kinetic parameters

Assuming that the microalgae growth can be modeled by a first order dynamic equation:

$$dy/dt = \mu X \quad (1)$$

Integrating and re-arranging Eq. 1, the growth coefficient also called specific growth rate (μ, d^{-1}) can be calculated using the Eq. 2:

$$\mu = \frac{\ln(X_2 / X_1)}{t_2 - t_1} \quad (2)$$

where X_1 and X_2 were the microalgae concentration (g L^{-1}) on days t_1 and t_2, respectively[13]. The biomass productivity rate, also called linear growth (p), is estimated according to Eq. 3:

$$p = \frac{X_2 - X_1}{t_2 - t_1} \quad (3)$$

where p (g L^{-1} d^{-1}). Since no organic carbon source is available in culture medium, the CO_2 biofixation rate can be indirectly calculated by the carbon content and biomass productivity rate, according to Eq. 4[13]

$$R_{CO2} = Cc \times p \times (M_{CO2}/M_C) \quad (4)$$

Therefore, the rate of CO_2 biofixation per initial inoculation mass of microalgae can be determined by Eq. 5

$$r_{CO2} = R_{CO2} / Xo \quad (5)$$

where R_{CO2} [g_{CO2} L^{-1} d^{-1}] is the biofixation rate and r_{CO2}[g_{CO2} g^{-1} dry cell].

The average cell carbon content Cc [g_C g^{-1} dry cell] ratio measured experimentally was 0.59 g carbon g^{-1} dry cell weight, according to the measurement using an elemental analyzer, and M_{CO2} M_C^{-1} represents the molecular weight of CO_2 and C[4,14], respectively.

The efficiency of CO_2 removal was calculated as follows: 100 × $(CO_2)_{input}$.- $(CO_2)_{output}/(CO_2)_{input}$, where CO_{2input} is the initial and $(CO_2)_{output}$ the stream gas off the bioreactor[4].

Empirical microbial growth kinetic models were explored to describe the impact of environmental factors on specific microbial growth of *S. platensis* and *S. platensis* adapted. Mönod model (Eq. 6).

$$\mu = \mu_{max} \frac{S}{Ks + S} \quad (6)$$

where *Ks* is the Mönod kinetic constant and *S*, CO_2 concentration or light intensity. When the substrate inhibits microbial growth at high concentrations, an optimum at which the highest specific growth rate occurs in this case, the Mönod model can be modified by Andrews model (Eq. 7).

$$\mu = \mu_{max} \frac{S}{Ks + S + S^2 / K_I} \quad (7)$$

where *Ks* and *S* are the same meaning as Mönod, and K_I is the inhibition constant.

The effect of the temperature on the maximum specific growth rate is based on Arrhenius model (1889), which implies an exponential increase in growth rate of the cells with rising the temperature. However, it is well recognized that the Arrhenius model fails once the temperature approaches the value of optimum activity, because it cannot represent the decline in rates at higher temperatures. Due to this limitation, alternative models have been proposed, which can predict the decline in rate following the optimum. Mayo model (Eq. 8) modified the Arrhenius equation based on the premise that the active fraction of the enzymes involved in the growth limiting reaction deceases when the temperature exceeds the optimum, this expression is also able to predict a decline in the maximum specific growth rate when the temperature exceeds the optimum[15].

$$\mu = \frac{A' e^{(-E_1 / RT)}}{1 + k e^{(-E2 / RT)}} \quad (8)$$

where *A'* and *k* are constants, E_1 is the activation energy for cellular multiplication, and E_2 is the activation energy for the thermal denaturalization process.

2.5 Statistical analysis

The experimental results were evaluated by comparing the specific growth rates of CO_2 biofixation by *S. platensis* and *S. platensis* adapted to salinity

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under different environmental conditions in photobioreactors and analysis of variance (ANOVA) of the kinetic parameters, significance was tested by Tukey at $p < 0.05$, using R software[16].

3. Results and discussion

3.1 Effect of salinity on the dry cell mass growth of S. platensis *under different environmental conditions*

Figure 2 gives the growth curve of dry cell mass of *S. platensis* cultivated in photobioreactors at normal salinity concentration for 12 days cultivation period at 2.5% CO_2 concentration, 25 °C and µmol m^{-2} s^{-1} dry cell mass achieves 4.3 g L^{-1}, which slowly declines when cultivation conditions change by increasing CO_2 concentration (5%), temperature (30 °C) and light intensity (150 µmol m^{-2} s^{-1}). The dry cell mass growth of *S. platensis* continued to decline even more and photosynthesis of *S. platensis* is inhibited at cultivation conditions of CO_2 concentration (10%), temperature (40 °C), and light intensity (200 µmol m^{-2} s^{-1}). Figure 3 shows the impact of salinity when *S. platensis* adapted to100 g L^{-1} NaCl (1.71 mol L^{-1}) is cultivated in the same way of *S. platensis*, for example, dry cell mass achieves 3.2 g L^{-1} for a 12 days cultivation period at 2.5% CO_2 concentration, 25 °C and 100 µmol m^{-2} s^{-1}, salinity-stress is usually accompanied by photoinhibition of photosynthesis[10]. Figure 4 illustrates the UV-visible spectrums of *S. platensis* and *S. platensis* adapted. An analysis of the spectrum from 600 to 800 nm shows the strongest band centered near 680 nm for *S. platensis*, which decreases and displaces the maximum to 675 nm for *S. platensis* adapted, due to the impact of salinity.

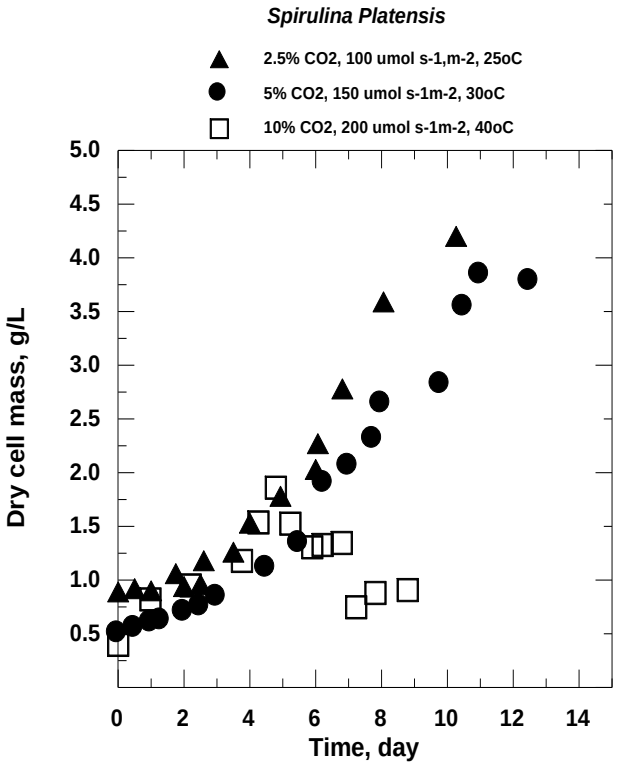

Figure 2. Dry cell mass of *S. platensis* grown under different CO_2 concentrations, temperatures and light intensities.
Adapted from "Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors", by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 69.

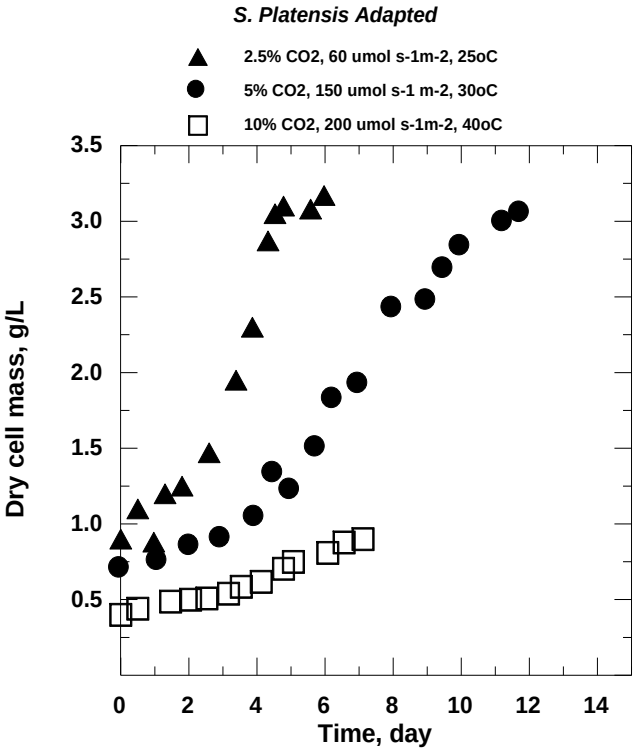

Figure 3. Salinity effect on dry cell mass of *S. platensis* (adapted) grown at 100 mg L^{-1} NaCl and under different CO_2 concentrations, temperatures and light intensities.
Adapted from "Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors", by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 69.

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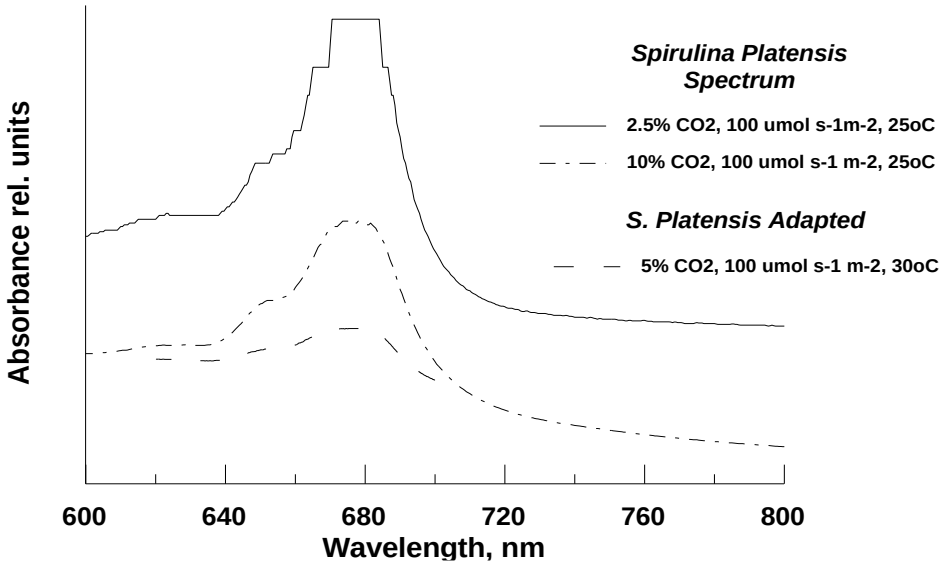

Figure 4. Impact of salinity on the UV-vis absorption of *S. platensis* and *S. platensis* adapted.Adapted from "Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors", by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 69.

3.2 Effect of Salinity on the specific growth rate (μ) and CO_2 biofixation rate (RCO_2)

Table 3 shows that during the 12 days cultivation period, the highest μ and R values of *S. platensis* (0.278 d^{-1}, 25.1 g_{CO2} m^{-3} h^{-1}) cultivated at 2.5% CO_2 was significantly higher ($p < 0.0012$) than 5% CO_2 or higher CO_2 concentration runs. In the same cultivation mode, the μ and R of *S. platensis* adapted decreases (0.15 d^{-1}, 13.9 g_{CO2} m^{-3} h^{-1}) because of the effect of salinity, in addition to the high CO_2 concentration of 5 to 10% runs, according to the results of Tab. 3. There was no significant difference between μ and R values of *S. platensis* between 20 and 25 °C runs, but there were significant differences ($p < 0.0012$) between μ and R values of *S. platensis* adapted in the same range of temperatures, these values were significantly lower when the cells were exposed to higher temperatures from 30 to 40 °C, respectively. When *S. platensis* were exhibited to 150 μmol s^{-1} m^{-2} μ and R values achieved higher values elevated light intensities e.g. 0.18 d^{-1}, 16.2 g_{CO2} m^{-3} h^{-1} than corresponding values at 100 and 60 μmol s^{-1} m^{-2}. But there was no significant difference between μ and R of *S. platensis* for 100 and 150 μmol s^{-1} m^{-2} runs.

Table 3. Specific growth rate (μ, d^{-1}); CO_2 biofixation rate (R, g_{CO2} m^{-3} h^{-1}) for *S. platensis* and *S. platensis* adapted growing in photobioreactors under different CO_2 concentrations, temperatures and light intensities.

Treatment	**μ / d^{-1}**		**R / g_{CO2} m^{-3} h^{-1}**	
ANOVA	*S. platensis*	*S. platensis Adapted*	*S. platensis*	*S. platensis Adapted*
Carbon dioxide, %, Pr(>F)	$2.90e^{-11}$***	$4.45e^{-2}$*	$3.00e^{-11}$***	$4.47e^{-2}$*
0.5	0.1149 c	*NA*	10.36 c,d	*NA*
2.5	0.2783 a	0.1540 b	25.08 a	13.88 b,c
5.0	0.1592 b,c	0.1105 b,c	14.34 b,c	10.38 c,d
7.5	0.0597 c,d	0.1045 b,c	5.38 d,e	9.42 d,e
10.0	0.0549 c,d	0.0501 d	4.94 e	4.53 e
Temperature, °C, Pr(>F)	$1.67e^{-5}$***	$8.68e^{-13}$***	$1.68e^{-5}$***	$8.64e^{-13}$***
12	0.0260 e	*NA*	2.32 e	*NA*
15	0.0477 d	0.0297 e	4.28 d,e	2.70 e

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20	0.1283 a,b	0.1070 b,c	11.56 b,c	9.70 c
25	0.1471 a,b	0.2022 a	13.26 b	18.23 a
				Continue…
30	0.1109 b	0.0978 c	9.99 b,c	8.83 c,d
40	0.0278 e	0.0493 d	2.51 e	4.45 d,e
Light, µmol $s^{-1} \times m^{-2}$, Pr (>F)	$2.49e^{-2}$*	$4.42e^{-3}$**	$2.40e^{-2}$*	$4.22e^{-3}$**
60	0.1129 b,c	0.1467 a,b	10.18 b,c	13.22 b
100	0.1413 a,b	0.1111 b,c	12.73 a,b	10.03 b,c
150	0.1800 a	0.1120 b,c	16.24 a	10.1 b,c
200	0.0734 d	0.0549 e	6.61 d	4.96 d,e

NA Not available
Signif. codes: 0 `***' 0.001 `**' 0.01 `*' 0.05 `.' 0.1 ` ' 1
Tukey multiple comparisons of means 95% family-wise confidence level

Conversely, the µ and R values of *S. platensis* adapted, increased as the cells were exposed to lower light intensity from 60 µmol s^{-1} m^{-2} (0.15 d^{-1}, 13.2 g_{CO2} m^{-3} h^{-1}) to 200 µmol s^{-1} m^{-2} (0.05 d^{-1}, 4. 96 g_{CO2} m^{-3} h^{-1}). But there were no significant differences between µ and R values of *S. platensis* adapted cultivated with 60 and 100 µmol s^{-1} m^{-2}, respectively, indicating that *S. platensis* adapted to salinity stress is less tolerant to growth at elevated light intensity and temperature, suggesting a decrease in photosynthetic activity of *S. platensis* adapted (Tab. 2).

3.3 Effect of salinity on the kinetics of S. platensis and S. platensis adapted

The relationship between the µ value and environmental cultivation parameters such as CO_2 concentration, temperature and light intensity in terms of experimental kinetic models (Monöd, Andrews and Mayo) and the impact of salinity is illustrated for *S. platensis* (Tab. 3) and *S. platensis* adapted (Tab. 4), respectively. The impact of light intensity on µ of *S. platensis*, in principle is described by Mönod well in the range of 60 to 150 µmol m^{-2} s^{-1} Eqs. 9 and 10 (Tab. 4). The maximum specific growth (μ_{max}) estimated (0.44 d^{-1}) is depleted as CO_2 concentration and temperature were increased. Under these conditions, μ_{max} decreased to 0.22 d^{-1} and Andrew's model described better the kinetics of *S. platensis* exposed to higher light intensity (Eqs. 11, 12 in Table 4 and Fig. 5). In fact, a decline in the photosynthetic activity was observed after four days of experiment run under 10% CO_2, 200 µmol m^{-2} s^{-1}, and 40 °C, suggesting these values as potential limiting factors of microalgae growth and photoinhibition[10]. The influence of light intensity on the kinetics of *S. platensis* adapted was stronger than the impact on the µ of pure culture *S. platensis* (see Tab. 5: Eqs. 19, 20 and Fig. 5), Andrew's model described this impact, μ_{max} dropped from 0.31 d^{-1} for 5% CO_2 and 25 °C to 0.22 d^{-1} for light intensities higher than 150 µmol m^{-2} $s^{-1}$and photoinhibition occurred when CO_2 and temperature increase to 10% and 40 °C, suggesting that in addition to light intensity salt stress affect its photosynthetic activity and may inhibit completely at 10% CO_2 and 40 °C. These results agree with Zeng *et al.*[10], who reported that the effect of salinity stress was stronger when cells of *S. platensis* were grown under higher light intensity 200 µmol m^{-2} s^{-1} and showed lower capacity of recovery in the photosynthetic activity after photoinhibition than lower light intensity (100 µmol m^{-2} s^{-1}) grown cells. This was attributed as a result of the fact that stressed cells have lower protein synthesis capacity and thus a slower repair mechanism.

Table 4. Experimental kinetic models and kinetic parameters of *S. platensis*.

Effect of light intensity	
$\mu = 0.0183\frac{I}{72.3+I}$; (25 °C, 2.5%,)	(9)
$\mu = 0.0119\frac{I}{80.2+I}$; (25 °C, 5.0%)	(10)
$\mu = 0.012\frac{I}{92.3+I+I^2/74.0}$; (30 °C, 7.5%)	(11)

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$\mu = 0.009\frac{I}{98.3 + I + I^2/62.3}$; (40 °C, 10%)	(12)
Continue…	
Effect of CO_2 Concentration	
$\mu = 0.028\frac{CO_2}{1.76 + CO_2 + CO_2^2/3.43}$; (25 °C, 150 μmol s^{-1} m^{-2})	(13)
$\mu = 0.021\frac{CO_2}{1.37 + CO_2 + CO_2^2/1.19}$; (30 °C, 100 μmol s^{-1} m^{-2})	(14)
$\mu = 0.017\frac{CO_2}{1.77 + CO_2 + CO_2^2/3.16}$; (40 °C, 200 μmol s^{-1} m^{-2})	(15)
Effect of Temperature	
$\mu = \frac{0.029e^{(-0.19/RT)}}{1 + 288.3e^{(-1.55/RT)}}$; (2.5%, 60 μmol s^{-1} m^{-2})	(16)
$\mu = \frac{0.020e^{(-0.20/RT)}}{1 + 304.2e^{(-1.50/RT)}}$; (5%, 100 μmol s^{-1} m^{-2})	(17)
$\mu = \frac{0.014e^{(-0.20/RT)}}{1 + 310.2e^{(-1.60/RT)}}$; (10%, 200 μmol s^{-1} m^{-2})	(18)

Table 5. Experimental kinetic models and kinetic parameters of *S. platensis* adapted.

Effect of light intensity	
$\mu = 0.013\frac{I}{94.3 + I + I^2/134.2}$; (25 °C, 5%)	(19)
$\mu = 0.009\frac{I}{100.83 + I + I^2/111.5}$; (40 °C, 10%)	(20)
Effect of CO_2 Concentration	
$\mu = 0.024\frac{CO_2}{2.05 + CO_2 + CO_2^2/2.55}$; (25 °C, 60 μmol s^{-1} m^{-2})	(21)
$\mu = 0.010\frac{CO_2}{2.08 + CO_2 + CO_2^2/2.27}$; (40 °C, 200 μmol s^{-1} m^{-2})	(22)
Effect of Temperature	
$\mu = \frac{0.028e^{(-0.19/RT)}}{1 + 327e^{(-1.59/RT)}}$; (2.5%, 60 μmol s^{-1} m^{-2})	(23)

The effect of CO_2 concentration on the kinetics of *S. platensis*, Eqs. 13, 14 and 15 (Tab. 4) and *S. platensis* adapted Eqs. 21 and 22 (Tab. 5), is depicted by Andrew's kinetic model and is illustrated in Fig. 6. The μ_{max} value estimated for 2.5% CO_2, 25 °C and 150 μmol m^{-2} s^{-1}, declined from 0.67 d^{-1} to 0.41 d^{-1} when CO_2 concentration increased in the range of 5 to 10% and the temperature and light intensity rose to 40 °C and 200 μmol m^{-2} s^{-1}, respectively, suggesting that CO_2 is a limiting factor that inhibited *S. platensis* growth particularly at 200 μmol m^{-2} $s^{-1}$and 40 °C. An optimal μ_{max} value for *S. platensis* adapted to salinity stress of 0.58 d^{-1} determined under the following environmental parameters 2.5 % CO_2 (25 °C and 60 μmol m^{-2} s^{-1}) declined to 0.24 d^{-1} when CO_2 concentration rose more than 5% (40 °C and 200 μmol m^{-2} s^{-1}), suggesting that CO_2 is a limiting factor of CO_2 biofixation in combination with high salt concentration. The effect of temperature on the kinetics of *S. platensis* (Eqs. 16, 17 and 18 in Tab. 4) and *S. platensis* adapted to salinity stress (Eq. 23 in Tab. 5) and depicted by Mayo's model (Fig. 7), the optimum temperature for cultivation of *S.*

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platensis occurred around 25 °C, 150 µmol s^{-1} m^{-2} and 2.5% CO_2 but it declined slowly as temperature increased along with CO_2 concentration and light intensity to a minimum µ values 10% CO_2 and 200 µmol m^{-2} s^{-1}. Likewise, as temperature decreased less than 15 °C, µ values decreased, indicating that the temperature is a limited growth factor under the influence of high CO_2 concentration and light intensity in spite the fact that *S. platensis* was characterized as thermophilic microalgae. The optimum temperature of *S. platensis* adapted occurred at around 25 °C, for 60 µmol m^{-2} s^{-1} and 2.5% CO_2 but declined slowly as the temperature increased. Despite the fact that *S. platensis* has been characterized as a thermophilic microalgae, the high salt concentration may have caused strong impact on the cells stress supported by high CO_2 concentration (10%) and light intensity (200 µmol m^{-2} s^{-1}) minimize µ values. Likewise, as temperature decreased less than 15 °C, µ values decreased, suggesting that the temperature is a limiting growth factor. In addition, other factors were shown, such as CO_2 concentration and light intensity in combination with high salt concentration affect CO_2 biofixation rate.

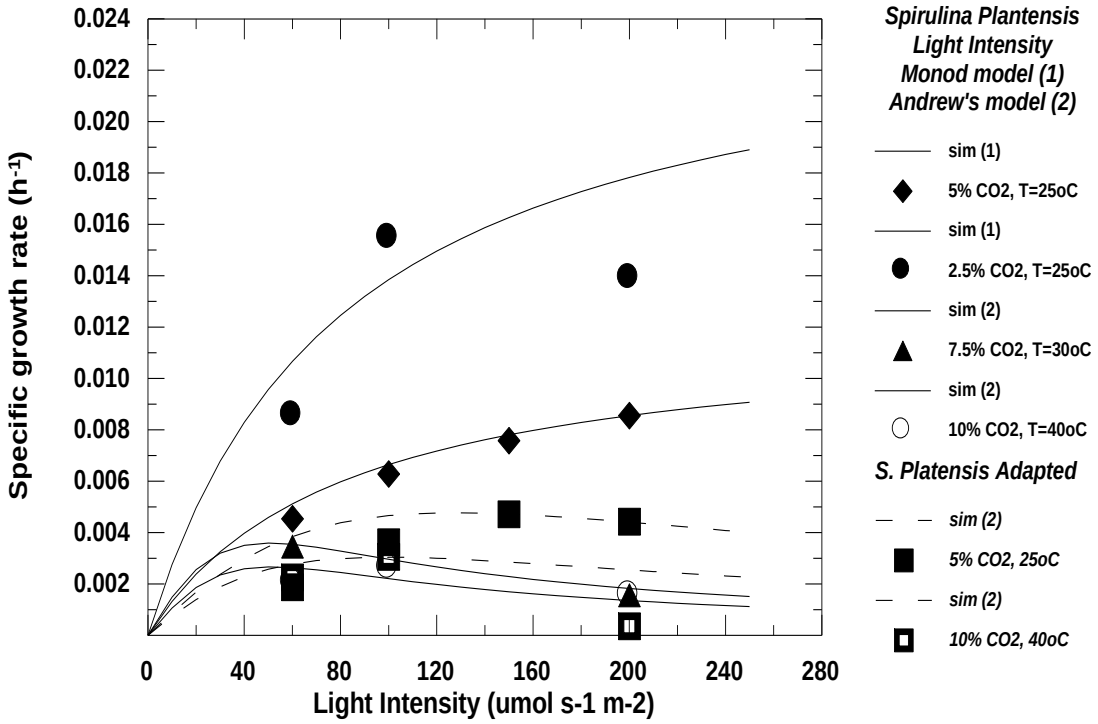

Figure 5. Effect of light intensity on the kinetics of *S. platensis* and *S. platensis* adapted to salinity stress at different CO_2 concentrations and temperatures.
Mönod model fit (solid line), Andrew's model fit (dash line). Adapted from "Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors", by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 71.

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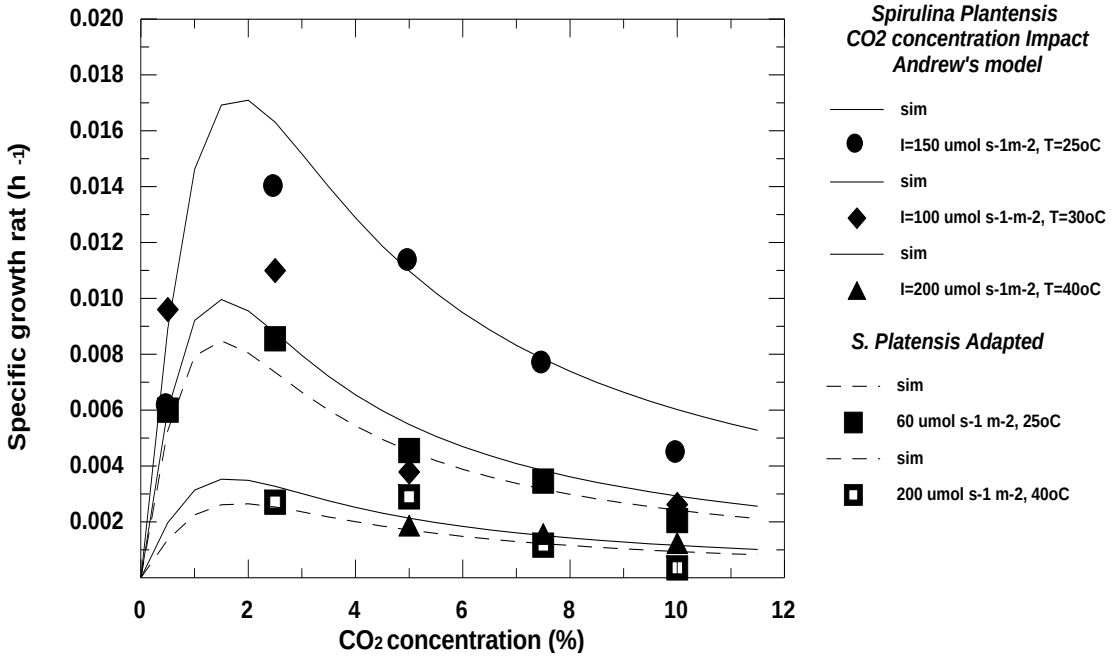

Figure 6. Effect of CO_2 concentration on the kinetics of *S. platensis* and *S. platensis* adapted to salinity stress at different light intensities and temperatures.
Adapted from "Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors", by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 71.

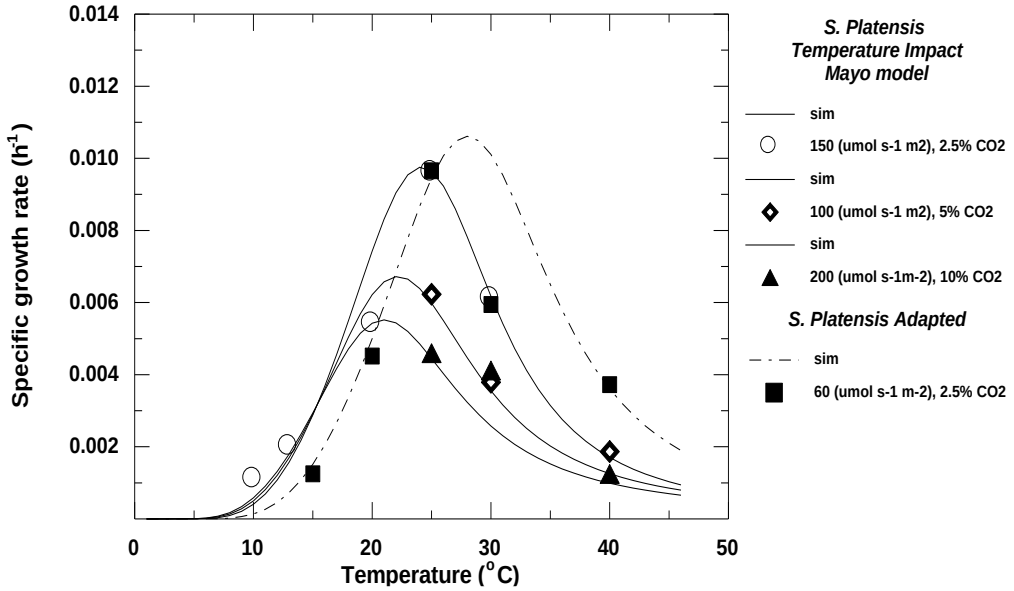

Figure 7. Effect of temperature on the kinetics of *S. platensis* and *S. platensis* adapted to salinity stress at different light intensities and CO_2 concentrations.
Adapted from "Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors", by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 72.

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Waste stream flue gases emitted from stationary sources such as power plants, industrial boilers, refineries and others, using fossil fuels for combustion and energy production produce from 4 -14% CO_2 and air in a closed space such as a space station or a submarine < 1% CO_2[2]. It has been reported that microalgae present one of the few technologies for the capture and utilization of CO_2[17]. The results indicate that both *S. platensis* and *S. platensis* adapted to high salt concentration can be used for CO_2 biofixation of flue gas emitted from stationary sources. In fact, maximum daily evaluation of the efficiency CO_2 removal by *S. platensis* during experiments up to ten days, achieved 92% at 0.5% CO_2, 99% at 2.5% CO_2, 84% at 5% CO_2, 89% at 7.5% and 88% at 10% CO_2, respectively. Corresponding values for *S. platensis* adapted being 96% at 2.5% CO_2, 90% at 5% CO_2, 78% at 7.5% CO_2 and 73% at 10% CO_2, respectively (See Appendix).

Small pH changes of *S. platensis* cell suspension observed during experiments from an initial pH (8.9) in the presence of $CO_2 \leq 2.5\%$ and temperatures ≤ 25 °C, after 24 h pH rose to 9.4 ± 0.1 and *S. platensis* adapted from an initial pH of 8.5 increased to 8.9 ± 0.1. However, in cultivation of *S. platensis* at $CO_2 > 5\%$ and temperatures > 25 °C the pH increased to ~~e~~ 9.1 ± 0.2 and *S. platensis* adapted to 9.2 ± 0.2, after 24 h. These results indicate that there was no formation of carbonic acid but no abrupt decline in pH due to *S. platensis* and *S. platensis* adapted were able to metabolize CO_2 because both microalgae were previously acclimatized to CO_2. *S. platensis* and *S. platensis* adapted were able to metabolize CO_2 because both microalgae were previously acclimatized to CO_2. Therefore, neither was formation of carbonic acid nor decline in pH.

In this study, the maximum mean CO_2 biofixation rate recorded for *S. platensis* was 25.1 g_{CO2} m^{-3} h^{-1} cultivated at 2.5% CO_2, 150 µmol m^{-2} s^{-1} and 25 °C, corresponding values for *S. platensis* adapted being 18.2 g_{CO2} m^{-3} h^{-1} for 2.5% CO_2, 60 µmol m^{-2} s^{-1} and 25 °C.

In a three-stage serial tubular photobioreactor were cultivated *Scenedesmus obiquus* and *Spirulina* sp. at 30 °C. It was found that, for *Spirulina* sp. the μ_{max} was 0.44 d^{-1}, 9.2 g m^{-3} h^{-1} with 6% CO_2, maximum daily CO_2 removal efficiency was 53.3% for 6% CO_2 and 45.6% for 12% CO_2, the corresponding values for *S. obliquus* being 28.1% for 6% CO_2 and 13.6% for 12% CO_2 runs[14]. Yun *et al.*[18] estimated 26.0 g_{CO2} m^{-3} h^{-1} biofixation rate value when *Chlorella vulgaris* was cultivated after adaptation in 5% CO_2 in wastewater supplemented with nutrients and without pH control at 15% CO_2, this value is comparable with the findings of this research and suggests that elevated CO_2 concentration may exert effects on the photoinhibitory behavior of the microalgae to different extents according to species[19]. The efficiency of CO_2 biofixation by these microalgae strains may have been due to its physiological conditions, such as potential of cell growth and ability of CO_2 metabolism. *S. platensis* was cultivated under different light intensities (100-200 µmol m^{-2} s^{-1}) at 35 °C and adapted to salinity stress up to 0.75 mol L^{-1}. It was reported that the cells grown in higher light intensity are less tolerant to salinity stress than those grown in lower light intensities, suggesting that salt stress enhances photoinhibition of photosynthesis through a direct effect on PSII reaction center[10]. The results demonstrated that *S. platensis* adapted to high salinity media 1.71 mol L^{-1} cultivated at 200 µmol s^{-1} m^{-2}, 40 °C and high CO_2 concentrations (7.5 and 10%) inhibit microalgae grow, show low photosynthetic activity and consequently photoinhibition[20]. Photoinhibition occurs when the photon flux absorbed by chloroplasts is extremely high, so the concentration of high energy electrons in the cells is too elevated to be consumed in the Calvin cycle. These electrons react with water to form hydrogen peroxide, which is highly harmful to sub-cellular structures and the cell itself, indicating that salinity enhances photoinhibition of photosynthesis through a direct effect on PSII reaction center, the reason for declination of PSII activity of cells under salinity stress remains open. Moreover, they believed that salinity stress induced damage or inactivation of PSII reaction center as it is in the case of photoinhibition of photosynthesis[10].

4. Conclusions

This study shows the potential CO_2 biofixation by *S. platensis* and *S. platensis* adapted to high salinity, 1.71 mol L^{-1} NaCl at laboratory scale in photobioreactors. In general, a better rate of CO_2 biofixation was achieved by *S. platensis* as indicated by its kinetic parameters and efficiency CO_2 removal, when compared to *S. platensis* adapted. The impacts of light intensity, CO_2 concentration and temperature on the specific growth rate followed the Mönod, Andrews and Mayo kinetic models. For *S. platensis* the highest dry cell mass concentration was 4.1 g L^{-1}, cultivated at 2.5% CO_2, 25 °C and 150 µmol m^{-2} s^{-1}, the highest rate of biofixation 25.1 g_{CO2} m^{-3} h^{-1} and the maximum specific growth (μ_{max}) was 0.44 d^{-1}- 0.67 d^{-1}. The corresponding values for *S. platensis* adapted were 3.2 g L^{-1} at 2.5% CO_2, 25 °C and 60 µmol s^{-1} m^{-2}, 18.2 g_{CO2} m^{-3} h^{-1}, and μ_{max}, 0.32 d^{-1} – 0.58 d^{-1}. This suggests that the impact

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of salinity in combination with environmental grow factors such as elevated CO_2 concentration, light intensity and temperature may exert effects on the photoinhibitory behavior of *S. platensis* adapted, provoking a CO_2 biofixation rate depletion. Therefore, the photosynthetic biofixation of CO_2 *S. platensis* and *S. platensis* adapted showed optimum values at 2.5% CO_2 and 25 °C, and more sensitivity to light intensity than *S. platensis*, suggesting that salinity enhanced photoinhibition of photosynthesis. The efficiency of CO_2 removal by *S. platensis* achieved 99%, whereas for *S. platensis* adapted 96%, both at 2.5% CO_2 concentration and small pH changes exhibited both microalgae cell suspension.

References

[1] National Oceanic and Atmospheric Administration, Trends in Atimospheric Carbon Dioxide. https://www.esrl.noaa.gov/gmd/ccgg/trends/weekly.html.

[2] Department of Energy, Carbon Capture R&D, Office of Fossil Energy, Washington. https://www.energy.gov/fe/science-innovation/carbon-capture-and-storage-research/carbon-capture-rd.

[3] Intergovernmental Panel on Climate Change. Climate Change 2007: Mitigation of Climate Change. Cambridge University Press, Cambridge, 2007. https://doi.org/10.1017/CBO9780511546013.

[4] Ramirez-Perez J. C., Algal Photobioreactor an Air emission Cleanup and Renewable Energy Technology Demonstration, Final Technical Report EPA Region 2 (2006) 71.

[5] Ramirez-Perez, J. C., Janes H. W., Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors, Habitation 12 (1) (2009) 65-77. https://doi.org/10.3727/154296610X12686999887328.

[6] Ramirez-Perez, J. C., Hogan, J. A., Strom, P. F., Inedible Biomass Biodegradation for Advanced Life Support Systems: II. Compost Quality and Resource Recovery, Habitation 11 (4) (2008) 163-172. https://doi.org/10.3727/154296608785908615.

[7] Ciferri, O., *Spirulina*, the edible microorganism, Microbiological and Molecular Biology Reviews 47 (4) (1983) 551-578. https://doi.org/10.1128/MMBR.47.4.551-578.1983.

[8] Becker, E. W., Microalgae Biotechnology and Microbiology, Cambridge University Press, Cambridge, (1994).

[9] Ravelonandro, P. H., Ratianarivo, D. H., Joannis-Cassan, C., Isambertc, A., Raherimandimby, M., Improvement of the growth of *Arthrospira* (*Spirulina*) *platensis* from Toliara (Madagascar): Effect of agitation, salinity, and CO_2 addition, Food and Bioproducts Processing 89 (3) (2011) 209-216. https://doi.org/10.1016/j.fbp.2010.04.009.

[10] Zeng, M.-T., Vonshak A., Adaptation of *Spirulina platensis* to salinity-stress, Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 120 (1) (1998) 113-118. https://doi.org/10.1016/S1095-6433(98)10018-1.

[11] Maeda, K., Owada, M., Kimura, N., Omata, K., Karube, I. 1995. CO_2 fixation from the flue gas on coal-fired thermal power plant by microalgae. Energy Conversion and Management 36 (6-9) (1995) 717-720. https://doi.org/10.1016/0196-8904(95)00105-M.

[12] Matsumoto, H., Hamasaki, A., Sioji, N., Ikuta, Y., Influence of CO_2, SO_2 and NO in Flue Gas on Microalgae Productivity, Journal of Chemical Engineering of Japan 30 (4) (1997) 620-624. https://doi.org/10.1252/jcej.30.620.

[13] Zhao, B., Zhang, Y., Xiong, K., Zhang, Z., Hao, X., Liu, T., Effect of cultivation mode on microalgal growth and CO_2 fixation, Chemical Engineering Research and Design 89 (9) (2011) 1758-1762. https://doi.org/10.1016/j.cherd.2011.02.018.

[14] Morais, M. G., Costa J. A. V., Biofixation of carbon dioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor, Journal of Biotechnology 129 (3) (2007) 439-445. https://doi.org/10.1016/j.jbiotec.2007.01.009.

[15] Mayo, A. W., Effect of temperature and pH on the kinetic growth of unialgal *Chlorella Vulgaris* cultures containing bacteria, Water Environment Research 69 (1) (1997) 64-72. https://doi.org/10.2175/106143097X125191.

[16] R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, 2012. http://www.R-project.org/.

[17] Cheng, L., Zhang, L., Chen, H., Gao, C., Carbon dioxide removal from air by microalgae cultured in a membrane-photobioreactor, Separation and Purification Technology 50 (3) (2006) 324-329. https://doi.org/10.1016/j.seppur.2005.12.006.

[18] Yun, Y.-S., Lee, S. B., Park, J. M., Lee, C.-I., Yang, J.-W., Carbon Dioxide Fixation by Algal Cultivation Using Wastewater Nutrients, Journal of Chemical Technology and Biotechnology 69 (4) (1997) 451-455. https://doi.org/10.1002/(SICI)1097-4660(199708)69:4%3C451::AID-JCTB733%3E3.0.CO;2-M.

Eclética Química Journal, vol. 46, n. 1, 2021, 21-34
ISSN: 1678-4618
DOI: 10.26850/1678-4618eqj.v46.1.2021.p21-34

[19] Yang, Y., Gao, K., Effects of CO_2 concentrations on the freshwater microalgae, *Chlamydomonas reinhardtii*, *Chlorella pyrenoisa* and *Scenedesmus obliquus* (Clorophyta), Journal of Applied Phycology 15 (5) (2003) 379-389. https://doi.org/10.1023/A:1026021021774.

[20] Benemann, J. R., CO2 mitigation with microalgae systems, Energy Conversion and Management 38 (Suppl) (1997) S475-S479. https://doi.org/10.1016/S0196-8904(96)00313-5.

Appendix

Table 1A. *Spirulina platensis* efficiency of CO_2 removal.

R^{2*}	$[CO_2]_{in}$ (%)	$[CO_2]_{out}$ (%)	Eff (%)
1.00	0.5	0.02	96
1.00	0.5	0.03	94
0.92	0.5	0.04	92
0.87	0.5	0.05	90
0.89	0.5	0.05	90
0.97	2.5	0.05	98
0.97	2.5	0.02	99.2
0.97	5.01	0.7	86
0.94	5.13	1	80
0.98	5.13	0.65	87
0.89	5	0.95	81
0.94	5.01	0.7	86
0.84	5.01	1	80
0.85	5.12	0.65	87
0.85	7.5	0.95	87
0.25**	7.5	0.7	91
0.96	10	0.65	94
0.85	10	0.95	91
0.88	10	0.7	93
0.40**	10	1	90
0.56**	10	2.5	75
0.86	10	2.2	78
0.72**	10	0.7	93

* p-value = 0.001. ** p-value = 0.01.

Table 2A. *Spirulina platensis* adapted efficiency of CO_2 removal.

R^{2*}	$[CO_2]_{in}$ (%)	$[CO_2]_{out}$ (%)	Eff (%)
0.944	2.5	0.10	96
0.944	5.0	0.10	98
0.965	7.5	0.30	96
0.828	10.0	2.00	80
0.792	10.0	3.00	70
0.969	2.5	0.10	96
0.504**	5.0	0.05	99
0.6236**	7.5	3.00	60
0.971	10.0	3.00	70
0.856	5.0	0.10	98
0.906	5.0	0.15	97
0.837	5.0	0.60	88
0.897	5.0	1.50	70
0.6236**	5.0	0.10	98
0.977	5.0	0.15	97
0.912	5.0	0.95	81
0.970	5.0	1.00	80
0.805	5.0	0.10	98

* p-value = 0.001. ** p-value = 0.01.

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Chemical profiles and antimicrobial activities of plants utilized in Brazilian traditional medicine

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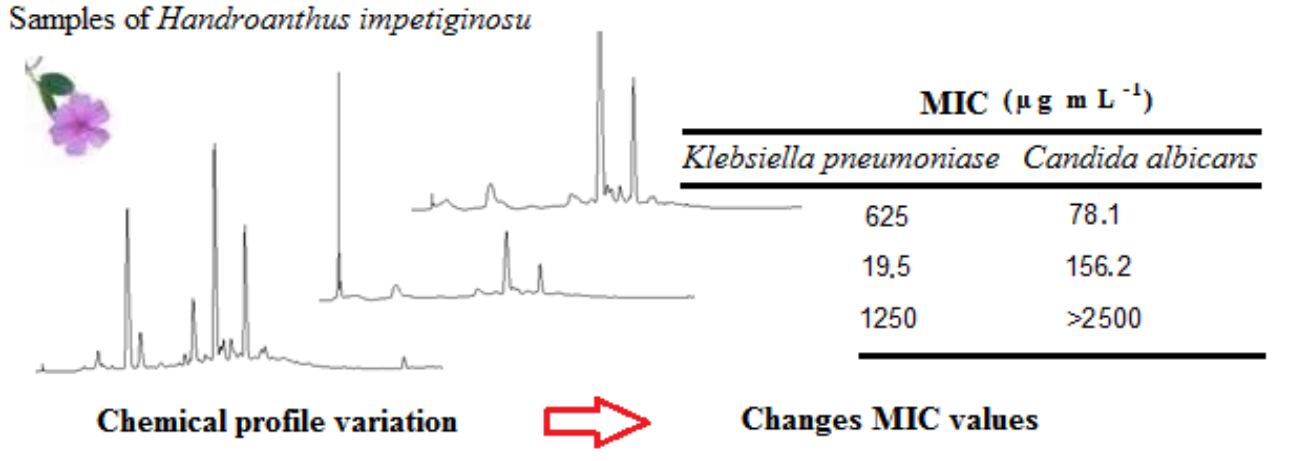
6. *Cnidosculus urens*

ABSTRACT: Medicinal plants are used for primary health care in many countries. In Brazil, there are hundreds of street markets selling a wide variety of herbs for medicinal purposes without quality control or scientific evidence**;** instead, their purported efficacy is based exclusively on empirical ethnobotanical knowledge. The present study evaluated the effectiveness of five medicinal plants widely utilized in Brazil to treat infections, as well as determined their chemical profiles. The results revealed that the five plants investigated **(***Anacardium occidentale* L., *Handroanthus impetiginosus* Mart., *Bumelia sartorum* Sw., *Zornia brasiliensis* Vogel and *Cnidosculus urens* Pohl**)** demonstrated moderate to strong antimicrobial activity against most fungi and bacteria tested, principally for infections caused by gram-negative bacteria. The extracts of four plants exhibited MIC of 19.5 µg mL^{-1} against the bacterium *Escherichia coli.* The results also confirmed that these five traditional medicinal plants are efficient and inexpensive alternative sources of substances to treat infections. The samples of the commercially marketed plants did not have consistent chemical compositions in at least one type of HPLC, GC/MS, UV or ^1H NMR analysis.



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1. Introduction

Since the beginning of human civilization, plants have been utilized for a wide array of purposes[1]. It is estimated that around 80% of the global population relies on medicinal plants for primary health care[1-3]. It is also estimated that more than 65% of commercial pharmaceutical preparations contain active ingredients from natural sources, 32% being natural compounds or derivatives[4]. Over a period of 30 years (1981 to 2014), 43.5% of the medicines in the world approved for the treatment of infections caused by bacteria, fungi, parasites and viruses were obtained from natural products[5]. Morphine, isolated from *Papaver somniferum* L., penicillin, obtained from fermentative processes of *Penicillium chrysogenum* Thom, and taxol, isolated from *Taxus brevifolia* Nutt., are among drugs developed from natural sources[6]. Despite the advantages of using medicinal plants, such as their lower cost, fewer side effects, greater protection and easier accessibility, there are still considerable risks to consumers due to problems of self-dosing, variability in the chemical standards and inflated marketing claims regarding herbs[7].

Brazil has the largest biodiversity in the world and 50% of plant species of the family Leguminosae have been reportedly used in folk medicine[8]. Hence, it is not surprising that street markets flourish selling myriad species of herbal and plant cures for an array of common ailments. Moreover, 66% of the Brazilian population has no full access to commercial medicines[9]. Indeed, medical anthropologists have verified the existence of time-tested ethnobotanical knowledge among cultures worldwide[10], including in Brazil[11,12]. There is no doubt about the importance of medicinal plants for the treatment and prevention of diseases. As part of the systematic study of phytochemicals and biological activity, previously were demonstrated the antidiarrheal property of *Solanum paniculatum*, L. roots, used in Brazil for culinary purposes and to treat gastric dysfunctions[13]. However, a lack of information about the origin, taxonomic identification, storage and chemical standardization of plants is a potential drawback to their safe consumption, posing possible health risks to users, particularly those in poor health[14]. Hence, the present study aimed to determine the chemical profile and antimicrobial potential of five medicinal plants (*Anacardium occidentale* L., *Handroanthus impetiginosus* Mart., *Bumelia sartorum* Sw., *Zornia brasiliensis* Vogel and *Cnidosculus urens* Pohl) popularly used in Brazil for the treatment of infections.

A. occidentale, known in Brazil as purple cashew, used in the form of tea and juice, is indicated for treatment of infections via oral administration and for wound healing by topical application[15,16]. The stems and flowers of *H. impetiginosus*, known as purple Ipê, are used to make poultices or concentrated as tea for the treatment of diseases caused by microorganisms[17-19]. Stems of *B. sartorum***,** known as *quixaba***,** are used in Northeast Brazil to prepare for treatment of various conditions, such as diabetes mellitus, inflammation, genital sores, colic, bruises, ulcers and others[20]. Extracts of leaves of *Z. brasiliensis*, known as *urinana*, are used for their molluscicidal, spasmolytic, muscle relaxant and anticonvulsant properties and reportedly possess antioxidant, antibacterial, cytotoxic, antinociceptive, anti-inflammatory and antitumor activities[21]. Extracts from roots of the white nettle, *C. urens*, are indicated for the treatment of cancer, inflammation, infection and dermatological lesions**,** besides being used for their antiseptic, expectorant, tonic, antispasmodic, diuretic, sedative and hemostatic activities[22].

2. Experimental

2.1 Plant material

2.1.1 Plant selection

The plant species were selected based on professed traditional ethnobotanical knowledge. Ten suppliers selling herbs at the São José market in the city of Recife (the state capital of Pernambuco, population 1,645,727; 2020) were interviewed by researchers and asked to identify the medicinal plants most frequently purchased by their customers for treating common infectious diseases. *Anacardium occidentale* (stems), *Handroanthus impetiginosus* (stems), *Bumelia sartorum* (stems), *Zornia brasiliensis* (leaves) and *Cnidosculus urens* (roots) were the plant species most frequently indicated by the herb vendors and thus, were selected for the study. Samples of these five plant species were purchased from three different medicinal plant suppliers in March 2018.

2.1.2 Obtaining extracts

The five selected plants were dried at 50 °C for 48 h. The plants were milled and extracted separately with ethanol (3 × 100 mL). The extract obtained was concentrated under reduced pressure at 45 ºC to yield the crude extract (Tab. 1). The extracts were subjected to chromatographic and spectrometric analysis (HPLC, GC/MS, UV-VIS e ^{1}H NMR).

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Table 1. Yields values of extracts obtained with ethanol from the five plants selected.

Samples		Dried plants (g)	Crude extracts (g)	Yielding (%)
A. occidentale	1Ao	32.3	0.7	2.0
	2Ao	19.1	0.6	3.0
	3Ao	40.3	0.8	2.0
H. impetiginosus	1Hi	43.4	1.0	2.2
	2Hi	32.2	2.1	6.7
	3Hi	26.7	1.4	5.3
B. sartorum	1Ba	56.4	1.2	2.2
	2Ba	29.9	0.9	2.8
	3Ba	36.4	2.6	7.3
Zornia sp	1Zs	18.9	0.5	2.7
	2Zs	17.3	0.5	3.2
	3Zs	23.5	0.65	2.7
C. urens	1Cu	20.5	0.5	2.9
	2Cu	24.0	0.3	1.1
	3Cu	21.5	0.2	1.0

2.2 Instruments

GC/MS analysis were carried out using a Perkin Elmer, model Clarus 589/Clarus SQ 8S capillary column (cross linked 5% phenyl methyl silicone, 0.25 mm i.d. x 30 m, Palo Alto, CA, USA), with oven temperature programmed from 100 to 250 °C at 10 ºC min^{-1} rate and a carrier gas (helium) flow rate of 1 mL min^{-1}. IR spectra were measured in KBr pellets with a Varian infrared spectrometer. The VIS-UV analyses were carried out in an Agilent 8453 UV-Vis spectrophotometer in the interval from 200 to 480 nm, using 10-mm quartz cuvettes. ^{1}H NMR analyses were recorded at 300 MHz using a Varian Unity Plus equipment. Samples were dissolved in $CDCl_3$ with TMS as the internal standard. HPLC analyses of extracts and pure compounds were performed in a Shimadzu LC 20AT instrument using a Luna C_{18} reverse phase column (250 × 4.6 mm × 5 µm, Phenomenex) and eluted in gradient mode starting with 0.001 % formic acid/methanol (3:7) for 5 min, rising to 90% formic acid after 30 min, with diode array detector. TLC was performed on pre-coated silica gel 60 F_{254} plates. Spots were visualized under UV light (254 and 365 nm).

2.3 In vitro assay for antimicrobial activity

The antimicrobial assay with crude extracts of the five plants studied was evaluated against the gram-positive bacteria *Bacillus subtilis* (UFPEDA 86), *Enterococcus faecalis* (UFPEDA 138), the gram-positive bacteria *Escherichia coli* (UFPEDA 224) and *Klebsiella pneumoniae* (UFPEDA 396), as well as against the fungi *Candida albicans* (ATCC 1007) and *Candida krusei* (UFPEDA 1002). The microorganisms were maintained in nutrient agar (NA), stored at 4 °C. The antimicrobial activity evaluation was performed by determination of the values of minimum inhibitory concentrations (MIC), as previously reported[23].

3. Results and discussion

The chemical profiles of the ethanolic extracts of *A. occidentale* (stems), *H. impetiginosus* (stems), *B. sartorum* (stems), *Z. brasiliensis* (leaves) and *C. urens* (roots) marketed as medicinal plants in Recife, Pernambuco, Brazil and indicated for the treatment of common infectious diseases were obtained by HPLC, TLC, GC/MS, UV and ^{1}H NMR analysis. Based on the interpretations of the spectroscopic and chromatographic analyses (see Supplementary Material, Figures S1-S15), some plant samples showed qualitative and quantitative differences in the chemical profiles in at least one type of analysis. The chemical profiles of *B. sartorum* specimens had the greatest chemical similarity among the five plants analyzed. The ^{1}H NMR spectra of the ethanolic extracts of *B. sartorum* samples revealed signals from the region of 0.9 to 5.5 ppm as bassic acid. Previous isolates from *B. sartorum* root bark and bassic acid have demonstrated anti-inflammatory activity[24]. Despite the small variation in the chemical profiles among the *B. sartorum* samples, the antimicrobial potential of *B. sartorum* showed a variation of MIC values, especially for sample **3Ba**, which presented lower activity against the bacterium *Escherichia coli* and the fungus *Candida albicans* (Tab. 2).

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Table 2. Minimum inhibitory concentrations (MIC) values in μg mL^{-1}.

Plants	Samples	Gram-positive bacteria		Gram-negative bacteria		Fungi	
		E. faecalis	*B. subtilis*	*E. coli*	*K. pneumoniae*	*C. krusei*	*C. albicans*
A. occidentale	1Ao	312.5	312.5	156.2	19.5	312.5	1250
	2Ao	156.2	78.1	78.1	19.5	625	2500
	3Ao	312.5	312.5	19.5	19.5	78.1	1250
H. impetiginosus	1Hi	1250	625	19.5	1250	2500	>2500
	2Hi	1250	625	156.2	625	2500	78.1
	3Hi	78.1	19.5	78.1	19.5	2500	156.2
B. sartorum	1Ba	1250	625	19.5	625	39.0	>2500
	2Ba	625	625	19.5	39.0	39.0	39.0
	3Ba	1250	625	1250	312.5	>2500	625
Zornia sp	1Zs	1250	1250	625	625	>2500	>2500
	2Zs	1250	625	625	625	>2500	2500
	3Zs	1250	312.5	625	625	>2500	78.1
C. urens	1Cu	1250	625	625	2500	>2500	>2500
	2Cu	1250	625	19.5	625	19.5	625
	3Cu	1250	625	19.5	625	19.5	625

The chemical profile of *H. impetiginosus* sample **1Hi** presented a difference when compared to the chemical profiles of samples **2Hi** and **3Hi**, mainly in the HPLC and UV analyses. *H. impetiginosus* exhibited strong antimicrobial activity with MIC of 19.5 μg mL^{-1} against the bacteria *E. coli*, *B. subtilis* and *K. pneumonia*, but the MIC values varied among the samples due to their different chemical profiles.

The chemical profiles of ethanolic extracts of the *Z. brasiliensis* samples showed differences when analyzed by HPLC, UV and ^{1}H NMR, especially sample **2Zs**. It was observed in the ^{1}H NMR spectrum of the *Z. brasiliensis* extract the presence of doublets at δ 7.6 and 7.30 ppm, an intense singlet from the methoxy group at δ 3.9 ppm, while the UV spectrum showed absorption in the 380 to 560 nm. These signals indicate the presence of flavonoids in the extract. Flavonoids as chalcones and flavones have been previously reported of *Z. brasiliensis* tissues, and flavone 7-methoxyflavone isolated from the aerial exhibited antinociceptive activity[25]. Among the species of plants studied here, *Z. brasiliensis* presented the least antimicrobial potential against all microorganisms tested.

The chromatograms obtained by HPLC of the three *C. urens* extract samples showed a major peak at t_r 40 min. The chemical profiles showed qualitative and quantitative differences, mainly the chromatogram of the **1Cu** sample. It was inactive against the fungi *C. albicans* and *C. krusei*, as well as presenting weak activity against all bacteria tested. The samples **2Cu** and **3Cu,** on the other hand, showed better results with strong activity against the gram-negative bacterium *K. pneumoniae* and the fungus *C. krusei*, with MIC of 19.5 μg mL^{-1}. The ^{1}H NMR spectra of *C. urens* extracts did not indicate the presence of hydrogen signals for aromatic compounds but showed standard signals of triterpenoids between δ 5.0 and 0.8 ppm, identical to the triterpenoid signals previously obtained for ethanolic extracts of *C. urens*[26].

Extracts from the *A. occidentale* samples exhibited strong antimicrobial activity against the bacteria tested, mainly against the gram-negative bacteria *K. pneumonia* and *E. coli*, corroborating previous studies which demonstrated the antimicrobial potential of *A. occidentale*[27,28]. The ^{1}H NMR analysis of *A. occidentale* samples revealed chemical signals characteristic of flavonoids and benzoic acid derivatives, such as quercetin, kaempferol, rhamnetin and 2-hydroxy-6-pentadecylbenzoic acid, previously observed in extracts of *A. occidentale* tissues[29,30].

4. Conclusions

In summary, the five medicinal plants popularly indicated for treating common infectious diseases in Recife, Brazil, showed moderate to strong antimicrobial activity against most of the fungi and bacteria tested, principally gram-negative bacteria, responsible for most infection-related deaths.

The results confirmed that these five traditional medicinal plants are efficient low-cost sources of extracts to treat infections, especially for the 5.1 million of Brazilians living in abject poverty[31] who have limited access to conventional medicines, as well as for other people seeking natural cures. The study also revealed that samples of the commercially marketed plants failed to have a consistent chemical

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composition in at least one type of HPLC, GC/MS, UV or ^{1}H NMR analysis. Another drawback noted was the lack of information about the authenticity of herbs on the packaging. The samples' variability suggests a need for more rigorous quality control of informally marketed herbal medicines in this setting to avoid potential risks to consumers' health.

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References

[1] Elekwa, I., Ugbogu, A. E., Okereke, S. C., Okezie., E., A Review of Selected Medicinal Plants with Potential Health Benefits in South-Eastern Nigeria, International Journal of Pharmaceutical and Chemical Sciences 6 (4) (2017) 162-171.

[2] Albuquerque, U. P., Hanazaki, N., As pesquisas etnodirigidas na descoberta de novos fármacos de interesse médico e farmacêutico: fragilidades e perspectivas, Revista Brasileira de Farmacognosia 16 (Suppl) (2006) 678-689. https://doi.org/10.1590/S0102-695X2006000500015.

[3] Pattanaik, C., Reddy, C. S., Medicinal Plant Wealth of Local Communities in Kuldiha Wildlife Sanctuary, Orissa, India, Journal of Herbs, Spices & Medicinal Plants 14 (3-4) (2008) 175-184. https://doi.org/10.1080/10496470802598800.

[4] Newman, D. J., Cragg, G. M., Natural Products as Sources of New Drugs over the 30 Years from 1981 to 2010, Journal of Natural Products 75 (3) (2012) 311-335. https://doi.org/10.1021/np200906s.

[5] Newman, D. J.; Cragg, G. M., Natural Products as Sources of New Drugs from 1981 to 2014, Journal of Natural Products 79 (3) (2016) 629-661. https://doi.org/10.1021/acs.jnatprod.5b01055.

[6] Barreiro, E. J., Fraga, C. A., M., Química Medicinal: As Bases Moleculares da Ação dos fármacos, Artmed, Porto Alegre 3rd ed., 2015.

[7] Yevale, R., Khan, N., Jagtap, P., Kale, M., A review on Herbal Medicine: Current Scenario, European Journal of Biomedical and Pharmaceutical Sciences 3 (4) (2016) 109-120. https://storage.googleapis.com/journal-uploads/ejbps/article_issue/volume_3_april_issue_4/1459406264.pdf.

[8] Souza, E. N. F., Williamson, E. M., Hawkins, J. A., Which Plants Used in Ethnomedicine Are Characterized? Phylogenetic Patterns in Traditional Use Related to Research Effort, Frontiers in Plant Science 9 (2018) 834. https://doi.org/10.3389/fpls.2018.00834.

[9] Mazzari, A. L. D. A., Prieto, J. M., Herbal medicines in Brazil: pharmacokinetic profile and potential herb-drug interactions, Frontiers in Pharmacology 5 (2014) 162. https://doi.org/10.3389/fphar.2014.00162.

[10] Urso, V., Signorini, M. A., Tonini, M., Bruschi, P., Wild medicinal and food plants used by communities living in *Mopane* woodlands of southern Angola: Results of an ethnobotanical field investigation, Journal of Ethnopharmacology 177 (2016) 126-139. https://doi.org/10.1016/j.jep.2015.11.041.

[11] Conde, B. E., Ticktin, T., Fonseca, A. S., Macedo, A. L., Orsi, T. O., Chedier, L. M., Rodrigues, E., Pimenta, D. S., Local ecological knowledge and its relationship with biodiversity conservation among two Quilombola groups living in the Atlantic Rainforest, Brazil, PLoS One 12 (11) (2017) e0187599. https://doi.org/10.1371/journal.pone.0187599.

[12] Almeida, C. F. C. B. R., Albuquerque, U. P., Uso e conservação de plantas e animais medicinais no Estado de Pernambuco (Nordeste do Brasil): um estudo de caso, Interciencia 27 (6) (2002) 276-285. https://www.redalyc.org/pdf/339/33906902.pdf.

[13] Tenório, J. A. B., Monte, D. S., da Silva T. M. G., Silva, T. G., Ramos, C. S., *Solanum paniculatum* root extract reduces diarrhea in rats, Revista Brasileira de Farmacognosia 26 (3) (2016) 375-378. https://doi.org/10.1016/j.bjp.2016.02.003.

[14] Ghorani-Azam, A., Sepahi, S., Riahi-Zanjani, B., Ghamsari, A. A., Mohajeri, S. A., Balali-Mood, M., Plant toxins and acute medicinal plant poisoning in children: A systematic literature review, Journal of Research in Medical Sciences 23 (2018) 26. https://www.jmsjournal.net/temp/JResMedSci23126-6450134_175501.pdf.

[15] Njinga, R. L., Jonah, S. A., Gomina, M., Preliminary investigation of naturally occurring radionuclides in some traditional medical plants used in Nigeria, Journal of Radiation Research and Applied Sciences 8 (2) (2015) 208-215. https://doi.org/10.1016/j.jrras.2015.01.001.

[16] Olaniyan, M. F., Cholesterol Lowering Effect of Cashew Leaf (*Anacardium occidentale*) Extract on Egg Yolk Induced Hypercholesterolaemic Rabbits, Scholars Academic Journal of Biosciences 4 (10B) (2016) 886-891. https://www.edouniversity.edu.ng/oerrepository/articles/cholesterol_lowering_effect_of_cashew_leaf_anacardium_occid

entale_extract_on_egg_yolk_induced_hypercholesterolaemic_rabbits.pdf.

[17] Park, J. G., Yi, Y.-D., Han, S. Y., Hong, Y. H., Yoo, S., Kim, E., Jeong, S.-G., Aravinthan, A., Baik, K. S., Choi, S. Y., Kim, J.-I, Son, Y.-J., Kim, J.-H, Cho, J. Y., Tabetri™ (*Tabebuia avellanedae* Ethanol Extract) Ameliorates Atopic Dermatitis Symptoms in Mice, Mediators of Inflammation 2018 (2018) 9079527. https://doi.org/10.1155/2018/9079527.

[18] Suo, M., Isao, H., Kato, H., Takano, F. Ohta, T., Anti-inflammatory constituents from *Tabebuia avellanedae*, Fitoterapia 83 (8) (2012) 1484-1488. https://doi.org/10.1016/j.fitote.2012.08.014.

[19] Coelho, J. M., Antoniolli, A. B., Silva, D. N., Carvalho, T. M. M. B., Pontes, E. R. J. C., Odashiro, A. N., O efeito da sulfadiazina de prata, extrato de ipê-roxo e extrato de barbatimão na cicatrização de feridas cutâneas em ratos, Revista do Colégio Brasileiro de Cirurgiões 37 (1) (2010) 045-051. https://doi.org/10.1590/S0100-69912010000100010.

[20] Sampaio, T. P. D., Cartaxo-Furtado, N. A. O., Medeiros, A. C. D., Alves, H. S., Rosalen, P. L., Pereira, J. V., Antimicrobial Potential of Plant Extracts and Chemical Fractions of *Sideroxylon obtusifolium* (Roem. & Schult.) T.D. Penn on Oral Microorganisms, The Journal of Contemporary Dental Practice 18 (5) (2017) 392-398. https://www.thejcdp.com/doi/JCDP/pdf/10.5005/jp-journals-10024-2053.

[21] Costa, E. V., Menezes, L. R. A., Rocha, S. L. A., Baliza, I. R. S., Dias, R. B., Rocha, C. A. G., Soares, M. B. P., Bezerra, D. P., Antitumor Properties of the Leaf Essential Oil of *Zornia brasiliensis*, Planta Medica 81 (7) (2015) 563-567. https://doi.org/10.1055/s-0035-1545842.

[22] Silva, T. C, Silva, J. M., Ramos, M. A., What Factors Guide the Selection of Medicinal Plants in a Local Pharmacopoeia? A Case Study in a Rural Community from a Historically Transformed Atlantic Forest Landscape, Evidence-Based Complementary and Alternative Medicine 2018 (2018) 2519212. https://doi.org/10.1155/2018/2519212.

[23] Silva, A. G. M., Silva, M. W. F., Bezerra, G. B., Ramos, C. S., The first report of chemical and biological study of essential oil from *Begonia reniformis* leaf (Begoniaceae), Eclética Química Journal 42 (1) (2017) 60-64. https://doi.org/10.26850/1678-4618eqj.v42.1.2017.p60-64.

[24] Almeida, R. N., Barbosa Filho, J. M., Naik, S. R., Chemistry and pharmacology of an ethanol extract of *Bumelia sartorum*, Journal of Ethnopharmacology 14 (2-3) (1985) 173-85. https://doi.org/10.1016/0378-8741(85)90085-6.

[25] Nascimento, Y. M., Abreu, L. S., Lima, R. L., Silva, A. D. S., Costa, V. C. O., Melo, J. I. M., Scotti, M. T., Sobral, M. V., Araujo, S. S., Gomes Filho, M. A., Zornioside, a dihydrochalcone *C*-glycoside, and other compounds from *Zornia brasiliensis*, Revista Brasileira de Farmacognosia 28 (2) (2018) 192-197. https://doi.org/10.1016/j.bjp.2018.02.003.

[26] Bhattacharyya, J., Barros, C. B., Triterpenoids of *Cnidosculus urens*, Phytochemistry 25 (1) (1985) 274-276. https://doi.org/10.1016/S0031-9422(00)94550-3.

[27] Madda, D. R., Lavanya M., Pola, S., Sandeep, B. V., Assessment of biological properties of two south Indian medicinal plants of *Bacopa monnieri* and *Anacardium occidentale*, International Journal Bioassays 5 (9) (2016) 4899-4905. https://www.ijbio.com/articles/assessment-of-biological-properties-of-two-south-indian-medicinal-plants-of-bacopa-monnieri-and-anacardium-occidentale.pdf.

[28] Santos, G. H. F., Silva, E.; B., Silva, B. L., Sena, K. X. F. R., Lima, C. S. A., Influence of gamma radiation on the antimicrobial activity of crude extracts of *Anacardium occidentale* L., Anacardiaceae, rich in tannins. Revista Brasileira de Farmacognosia 21 (3) (2011) 444-449. https://doi.org/10.1590/S0102-695X2011005000045.

[29] Kannan, V. R., Sumathi, C. S., Balasubramanian, V., Ramesh, N., Elementary Chemical Profiling and Antifungal Properties of Cashew (*Anacardium occidentale* L.) Nuts, Botany Research International 2 (4) (2009) 253-257.

[30] Paramashivappa, R., Kumar, P. P., Vithayathil, P. J., Rao, A. S., Novel Method for Isolation of Major Phenolic Constituents from Cashew (*Anacardium occidentale* L.) Nut Shell Liquid, Journal of Agricultural and Food Chemistry 49 (5) (2001) 2548-2551. https://doi.org/10.1021/jf001222j.

[31] Tronco, G. B., Ramos, M. P., Linhas de pobreza no Plano Brasil Sem Miséria: análise crítica e proposta de alternativas para a medição da pobreza conforme metodologia de Sonia Rocha, Revista de Administração Pública 51 (2) (2017) 294-311. https://doi.org/10.1590/0034-7612162786.

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Analytical procedures for the determination of estrogen compounds in a surface water reservoir in Southeast Brazil

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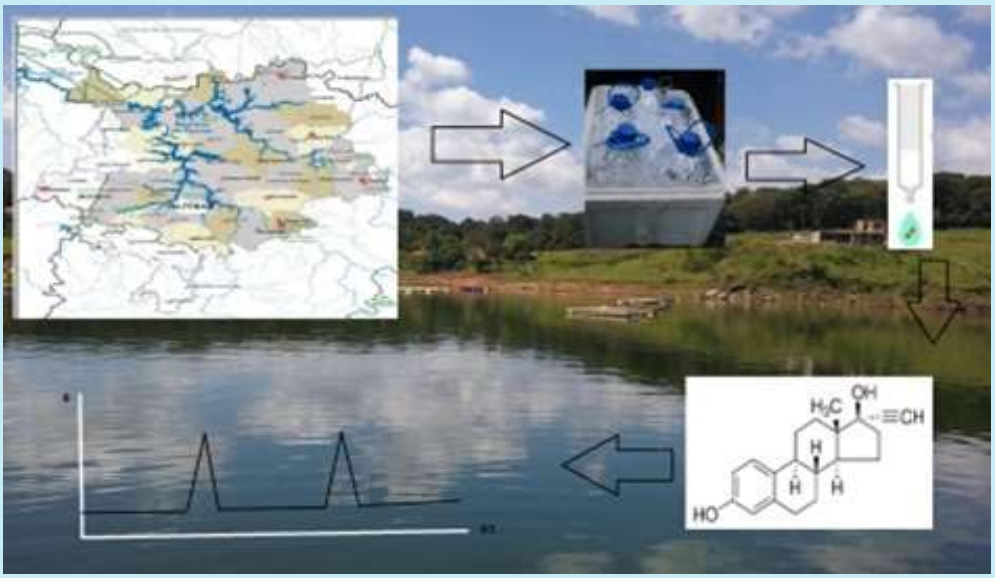
ABSTRACT: In this study, an analytical methodology was validated to determine and quantify four estrogen hormones using high-performance liquid chromatography (HPLC) with detections by diode array detector (DAD) and by fluorescence detector (FLD). For validation of the method, the following parameters were evaluated: linearity, selectivity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness. Environmental samples were preconcentrated using solid phase extractions and for that, an experimental design was planned to determine the best recovery conditions by varying cartridge types, flow of eluent, pH of the samples, and eluting solvent. Five surface water sampling campaigns were carried out in five different sites of Furnas Reservoir over the months of December 2015 and May 2016. Sample point 1 was located near the sewage treatment plant of the city of Alfenas - MG, while sample point 5 was the most distant from this location. All estrogens, except for E1, were found in all water samples of at least one of the sampling sites. The concentrations of E3, E2 and EE2 ranged from 11-366, 63-422 and 75-9998 ng L^{-1}, respectively. These results are consistent with several studies published in the scientific literature.



In this study, an analytical procedure was developed for the determination of estrogen compounds in water samples from Furnas reservoir, Alfenas - Minas Gerais (Brazil)

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1. Introduction

Water is intrinsically linked to development in all societies and cultures. However, developments in agriculture, power generation and industry have brought major impacts to water resources[1]. Despite ~71% of Earth's surface being covered by water, about 97.5% of this water is either saltwater, in oceans and seas, or unfit for human consumption. From the remaining 2.5%, which constitutes freshwater, two thirds are stored in glaciers and polar ice caps. Therefore, only a fraction (~0.77%) of Earth's water is available for human consumption and is found in rivers, lakes, groundwaters, soils, atmosphere (humidity) and within the biota[2]. The continuous environmental degradation associated with the increase of human population (currently estimated in 7.2 billion and projected to reach 9-10 billion by 2050) suggests a significant decrease in life quality in a near future[3,4]. Because of the anthropogenic activity, a large volume of water, which would be available for consumption, is becoming contaminated with a large variety of toxic compounds. Among these pollutants, the Emerging Contaminants (EC) have been attracting a lot of attention from the scientific community. They are defined as any chemical compound present in a variety of commercial products, such as personal care, agrochemical, medicines for human and veterinary uses, food packages or any microorganisms found in environmental matrices that are not monitored nor regulated; however, pose potential risk to human health and environment safety[5-10]. A variety of EC are known as Endocrine Disruptors Compounds (EDC). These substances are of special concern because they interfere with the endocrine system, blocking or minimizing normal hormone functions, affecting synthesis or metabolism of many organisms[11-15]. The 17α-ethynylestradiol (EE2) is considered a potent endocrine disruptor[15]. This synthetic substance is used as a contraceptive or in hormone replacement therapies, it is often excreted via urine and feces by individuals taking the medication, eventually reaching water compartments[16].

A variety of analytical methods and techniques can be used to detect and measure EDC from environmental matrices. The extraction approaches used more often are pressurized liquid extraction (PLE), quick-easy-cheap-effective-rugged-safe (QuEChERS), microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE), stir-bar-sorptive extraction (SBSE), solid phase microextraction (SPME) and liquid-liquid extraction (LLE). For analyte enrichment and sample cleanup, solid phase extraction (SPE) with C18 cartridges[17] are widely used. While for trace analysis of EDC, gas chromatography (GC) and liquid chromatography (LC)[17] are the dominant techniques.

Furnas is one of Brazil's largest water reservoirs with a flood area of 1,440 square kilometers, serving a population of approximately 800,000 inhabitants and irrigating 34 municipalities within the state of Minas Gerais[18]. This reservoir is also of paramount economic importance within these municipalities, supporting activities such as fish farming - with about 500 farmers and over 5,000 tanks within the vicinity of the reservoir[19].

The aim of this study was to develop an analytical methodology to determine the presence of estrogen hormones in the Furnas reservoir, located in the region of the city of Alfenas (MG).

2. Experimental

2.1 Reagents and solutions

All reagents used were of high purity with spectroscopic or HPLC grades. Deionized water (resistivity 18.2 MΩ cm 25 °C) was taken from a Direct-Q (Millipore®) purification system. The solvents methanol, acetonitrile (ACN), and ethyl acetate were purchased from Sigma-Aldrich (HPLC grade). Water and ACN filtrations were done using 0.45 μm pore-size nylon and cellulose ester membranes, which were acquired from Millipore® and Supelco Analytical, respectively. The estrogen standards estrone (E3), estriol (E1), 17β-estradiol (E2) and 17α-ethynylestradiol (EE2) were purchased from Fluka Analytical® with purity greater than 99%. Stock solutions of estrogens (100 mg L^{-1}) were prepared in methanol, dissolving 0.0100 g of each commercial standard in a 100.00 mL volumetric flask. Standard working solutions (10 mg L^{-1}) were prepared in ACN diluting 10.00 mL of the stock solution in a volumetric flask of 100.00 mL. After prepared, these solutions were stored in polyethylene bottles at 4 °C and used as calibration standards.

2.2 Validation of the analytical methodology

The analytical methodology used was validated based on several works available in the literature and in the evaluation of the following figure of merits: selectivity, linearity, precision, accuracy, limit of detection, limit of quantification and robustness[20-27].

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To confirm the selectivity of the method, estrogen standard solutions were prepared in acetonitrile at 1.00 mg L^{-1} and the spectral purity of each analyte was evaluated through chromatographic analysis. The linearity was verified by the linear coefficient of determination (r^2) resulting from the linear equation of nine and twelve points of the estrogen analytical curves, using DAD and FLD, respectively. For that, 12 mixed solutions were prepared from dilutions of the working solution in ACN at different concentrations (25 to 1000 µg L^{-1}). Solutions preparation were performed in triplicate and the external standardization method was used. The sensitivity of the method was determined by evaluating the angular coefficient value derived from the linear equation of each estrogen calibration curve.

The limits of detection and quantification were determined through the signal-to-noise ratio, considering the ratio 3:1 and 10:1 for LOD and LOQ, respectively.

Since no Certified Reference Material (CRM) is available for water contaminated with the estrogens under study, fortified distilled water samples with a mixed solution containing the four estrogens were prepared in triplicates, at concentrations of 0.40, 0.50 and 0.60 µg L^{-1}, extracted and injected into the HPLC system. Subsequently, method accuracy was evaluated through the recovery (R) values of the analytes. For determination of the real concentration, the value of the peak area of each analyte and the corresponding linear equation was used. The recovery (in percentage) of each analyte was determined from the mean real concentration values.

To determine the accuracy of the method, six mixed solutions of 500 µg L^{-1} were prepared and injected into the HPLC system by analyst 1. In the following day, the same procedure was performed by analyst 2. It is worth mentioning that all samples were analyzed in triplicate. The recovery values found were expressed in percentage and evaluated using F tests. The values for F ($F_{calculated}$) and F tabulated ($F_{tabulated}$) were compared with 95% of confidence level. The robustness of the method was evaluated by varying in ± 5% four chromatographic conditions: mobile phase composition, injection volume, flow rate of the mobile phase and temperature of the chromatographic column compartment. The obtained recovery values were compared to the ones achieved using normal conditions.

2.3 Optimization of solid phase extraction (SPE)

To verify the best SPE conditions for the analytes within the samples, a Box-Behnken design (BBD) was applied using Minitab 16® program. The planning was done with three levels: low (coded "-1"), central (code "0"), and high (coded "+1"), and four variables: cartridge types (Sorbline – C18, Chromabond – C18 and Strata-X – Polymeric resin), pH of water, flow rate of the eluent during extraction and elution solvent (Tab. 1). The total was 28 randomized experiments. During extractions, the conditions volume of water to be percolated (1 L), cartridge conditioning solvent (5.00 mL methanol) and cartridge wash solution (1.00 mL of water: 5% methanol) were invariable.

Table 1. Selected levels and factors in BBD.

Factors	**Levels**		
	-1	**0**	**+1**
Cartridge type	Sorbline	Chromabond	Strata-X
pH of water	4	6	8
Elution solvent	Methanol	Methanol: Ethyl acetate (1:1)	Ethyl acetate
Flow rate of mobile phase	3 mL min^{-1}	5 mL min^{-1}	10 mL min^{-1}

All extracted samples were prepared by fortifying 1 L of distilled water with 50.00 µL of mixed estrogen working solution at 10 mg L^{-1}. These samples were concentrated (1000 ×) to produce a solution of 0.5 mg L^{-1}. This concentration was considered the central point of the analytical curves.

Then, sample pH was adjusted according to the experimental design, the adsorbent present in the cartridges was conditioned with 5.00 mL of methanol and the fortified water was percolated by the cartridge. The next step was the removal of interferents from the cartridge using 1.00 mL of wash solution and finally the extract was eluted with 5.00 mL of elution solvent, according to the planned BBD. The eluates were dried in a water bath at approximately 70 °C, dissolved in 1.00 mL of ACN and sonicated in an ultrasonic bath for approximately 3 min for sample homogenization. Next, the samples were filtered through a 0.45 µm pore-size filter and injected into the HPLC system, following the established chromatographic conditions. After performing the 28 BBD experiments, the

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condition with the best performance (recovery) was selected.

2.4 HPLC analysis

The analyses were performed using an Agilent Technologies Infinity 1260 model chromatography system, consisting of a quaternary pump, an automatic injector, a column heating module, an ultraviolet detector with diode array and a molecular fluorescence detector. The acetonitrile and water, used as the mobile phase, were pre-filtered and degassed. In addition, an Eclipse Plus C8 (4.6 mm × 250 mm - 5 mm) chromatographic column was used. The optimum conditions for the chromatographic separation of these analytes are shown in Tab. 2.

Table 2. Optimized chromatographic conditions for analysis of estrogens using HPLC.

Run time	**18 minutes**
Mobile phase composition	water:acetonitrile - 1:1 (isocratic mode)
Flow rate of mobile phase	0.5 mL min^{-1}
Injection volume	10 µL
Temperature of the chromatographic column	40°C
DAD wavelength detector	285 nm
FLD wavelength detector	$\lambda_{excitation}$ – 230 nm e $\lambda_{emission}$ – 310 nm

2.5 Sampling sites

Samplings were spatially and seasonally carried out between December 2015 and May 2016 in Lake Furnas within the municipality of Alfenas - MG - Brazil (Fig. 1), according to instructions from the National Guide for Collection and Preservation of Samples[28] and with classifications of the National Water Agency[29].

Samples were collected from 5 different sites (P1, P2, P3, P4 and P5). P1 was the closest to Alfenas' Sewage Treatment Station (STS); while P5 was the most distant sampling site from this STS (Fig. 2). With the assistance of a fisherman, each sampling was taken every 1 km using a boat. Samplings were made between 11 am and 1 pm and the coordinates of the sites were identified using a GPS.

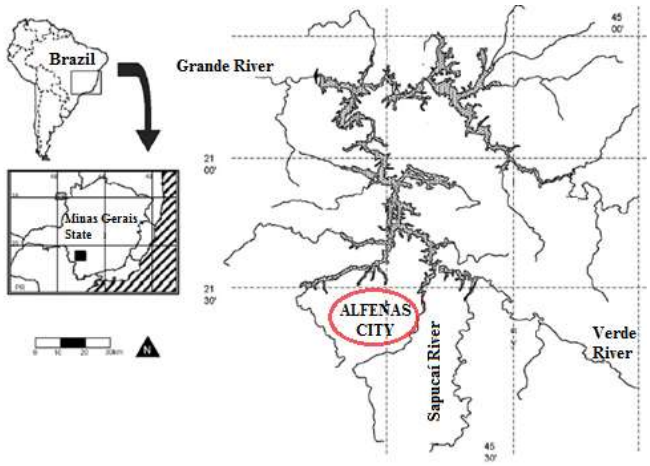

Figure 1. Map location of Lake Furnas and the municipality of Alfenas.
Source: Adapted from Prado et al. (2011)[30].

Figure 2. Sampling sites within Lake Furnas in the municipality of Alfenas.
Source: Google earth (2020)[31].

2.6 Samplings

Five liters of surface water were collected from each sampling site at a depth of ~30 cm, using a van Dorn bottle. The collected water was transferred to clean and acclimatized plastic bottles. Vials were sealed and transported in thermal containers to the laboratory where they were kept at 4 °C. Starting from the second sampling campaign, temperature, pH, conductivity and dissolved oxygen of the sampling site were measured *in situ* using a multiparameter probe (HI 9628 - pH/ORP/CE/DO).

2.7 Sample preparation

Samples from each sampling site were prepared in triplicate. A volume of 4 L was filtered several times through a Buchner funnel lined with qualitative filter

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paper (J. Prolab) and vacuum filtered in an enclosed glass system using 0.45 µm pore-size cellulose acetate membrane (Agilent Technologies). After filtration, the pH of the samples was adjusted to 8 using 0.5 mol L^{-1} ammonium hydroxide solution whenever necessary. Subsequently, solid phase extractions were performed and elution was analyzed in the HPLC system.

3. Results and discussion

3.1 Validation of the analytical methodology

The proposed method was selective for both detectors. Even though the four studied estrogens have similar structures, satisfactory peak separation could be noticed in their chromatograms.

The linearity and sensitivity of the method were determined using linear regression analyses of the calibration curve from each estrogen, where coefficient of determination (r^2) and angular coefficient values were evaluated, as presented in Tab. 3.

The methodology was linear for both detectors, the obtained r^2 were above 0.98 and, according to Brito *et al.*[24], represent a strong correlation between peak areas and analyte's nominal concentrations. The validated method was more sensitive using the fluorescence detector (FLD) than the ultraviolet detector (DAD), since the first presented higher angular coefficients values.

Table 3. Retention time, equation of the line, linearity, and sensitivity of estrogens under study.

Compounds	Retention time / min	Equation of the line	Linearity	Linear ranges / µg L^{-1}
Estriol	5.840	y = 15.388 × -237.26	0.9924	200-1000
17β-Estradiol	10.640	y = 16.366 × -526.74	0.9885	200-1000
17α-Ethynylestradiol	12.513	y = 15.473 × -682.62	0.9854	200-1000
Estrone	13.860	y = 17.307 × -693.63	0.9895	200-1000
Estriol*	5.897	y = 27608 × + 66759	0.9920	25-1000
17β-Estradiol*	10.699	y = 28561 × -79971	0.9955	25-1000
17α-Ethynylestradiol*	12.578	y = 28047 × -107662	0.9989	25-1000

***Estrogens detected by fluorescence detector (FLD).

According to the limits of detection (LOD) and of quantification (LOQ), the estrogens under study could be detected and quantified at concentrations of as low as 200 and 300 µg L^{-1} for DAD and 25 and 50 µg L^{-1} for FLD. However, this methodology predicts sample preconcentration using SPE (1000 ×); therefore, the LOD and LOQ of the method were 200 and 300 ng L^{-1} for DAD and 25 and 50 ng L^{-1} for FLD. The validated method using FLD can be considered more sensitive than when using DAD, since its values are lower.

The accuracy of the method was evaluated by mean recovery ($R_{average}$) and coefficient of variation (CV) values. For estrogens detected using DAD, $R_{average}$ ranged from 62.1-101.2% with CV of less than 15%. When using FLD, $R_{average}$ ranged from 80.0-105.6% with CV of less than 7.0%. These values are considered satisfactory, since the acceptable recovery values for samples at concentrations of ng L^{-1} are between 40-120% with CV of less than 45%[24].

The criteria employed to evaluate the method precision was based on the comparison of experimental and tabulated values of the F test for two sets of samples (n = 6 each set). Table 4 shows the mean recovery ($R_{average}$), coefficient of variation (CV), $F_{calculated}$ and $F_{tabulated}$ values for the datasets prepared by analysts 1 and 2.

The validated methodology proved to be accurate because, in all cases, $F_{cal} < F_{tab}$. There is no significant difference between the values of variance with 95% of credibility, since the in the values of $F_{tabulated}$, for a degree of freedom of 5, both denominator and numerator are equal to 5.05[22].

The method showed to be robust against variations in mobile phase composition, injection volume, flow rate of the mobile phase and chromatographic column compartment temperature. The mean recovery ($R_{average}$) values for the analytes using DAD were between 70.7-118.7% with a coefficient of variation (CV) of less than 10.6%. On the other hand, when using FLD the $R_{average}$ were between 91.0-108.8% with CV of less than 4.8%. The values obtained are within acceptable ranges; therefore, none of the alterations done to the chromatographic parameters showed significant effect on the recovery of the estrogens.

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Table 4. Average recovery ($R_{average}$), coefficient of variation (CV), $F_{calculated}$, and $F_{tabulated}$ values for the data sets prepared by analysts 1 and 2.

	Estrogen detected by DAD				Estrogen detected by FLD		
	E3	**E2**	**EE2**	**E1**	**E3**	**E2**	**EE2**
Analyst 1 $R_{average}$ (%) ± standard deviation	101 ± 7	112 ± 7	101 ± 7	104 ± 8	105 ± 1	107 ± 2	100 ± 2
Analyst 2 $R_{average}$ (%) ± standard deviation	109 ± 8	111 ± 10	108 ± 5	105 ± 8	108 ± 2	102 ± 2	102 ± 3
CV (%) Analyst 1	6.8	6.3	7.3	10.4	0.6	1.6	2.1
CV (%) Analyst 2	7.3	8.8	4.7	7.3	4.3	5.2	5.4
$F_{calculated}$	1.3	1.9	2.2	1.0	3.2	1.6	2.1
$F_{tabulated}$	5.05	5.05	5.05	5.05	5.05	5.05	5.05

3.2 Optimization of solid phase extraction (SPE) conditions

After performing the 28 BBD experiments, extracts were analyzed in a HPLC system and the average recovery of all detected analytes of each experiment was calculated. In some cases, the mean recovery exceeded the acceptable range for recovery of analytes at ng L^{-1} (40-120%), which may be related to co-elution of impurities presented within the cartridge.

The mean recovery data were used to obtain response surface graphs, corelating mean recovery values in the Z axis and two other variables (coded as -1, 0 and +1) in X and Y axes, using the software Statistica 2010. The related response surface graph variables were cartridge versus pH of water, cartridge versus elution solvent, cartridge versus flow rate of the eluent during the extraction, pH of water versus elution solvent, pH of water versus flow rate of the eluent during the extraction and elution solvent versus flow rate of the eluent during the extraction.

With regards to the flow rate of the eluent during the extraction versus cartridge type variable, it can be noticed that the lower the flow rate, the greater is the recovery, since the longer the sample stays in contact with the solid phase of the cartridge higher the chances of interactions between analytes and adsorbent phase.

Most BBD experiments presented recovery values within acceptable range for both detectors. However, according to the response surface graphs and the most satisfactory recovery values, it was opted to keep with the parameters used in the experiment number 5, which presents the following conditions: brand cartridge Chromabond C18-ec; pH of water equal to 8; Methanol: Ethyl acetate (1:1) as elution solvents; and flow rate of the eluent during the extraction of 3 mL min^{-1}.

3.3 Estrogens determination in Lake Furnas, Alfenas-MG

3.3.1 Some physical-chemical characteristics of water within the sampling sites

To test if Lake Furnas is in accordance with the environmental standards determined by Resolution National Council of the Environment (CONAMA) 357/2005[30], pH, conductivity, temperature, and dissolved oxygen within the vicinity of each sampling site were measured. Maximum and minimum values for each sampling sites are shown in Tab. 5.

According to the CONAMA Resolution 357/2005, Furnas Lake is classified as a class II river with pH values between 6.16 - 8.36. Therefore, within the permitted values (6.0 to 9.0).

The dissolved oxygen (OD) in water, which is essential for survival of aerobic organisms, according to this same legislation, should be higher than 5 mg L^{-1} [32]. In this term, the samplings that occurred in 04/28/16 and 05/19/16 presented values below the permitted level. This may be related to an environmental incident occurred in 05/01/16, where tons of tilapia, within tanks-nets, were found dead. According to the Environmental Military Police and professors from the University of Alfenas, the lack of sun-light and extended drought period contributed to the OD decrease, which may have caused the death of red algae and subsequent release of toxins to the surrounding water, aggravating fish mortality[33]. In a similar incident in 2013, also because of the drought, the level of the Lake lowered. After this period, with the increase of rain and temperature, there was a critical algae proliferation, causing drastic OD reduction, culminating in fish mortality again[34]. According to Tab. 5, temperatures within Lake Furnas varied from 23.98 to 29.21 °C, possibly indicating normal seasonal variations.

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According to the CETESB specifications[35], conductivity values higher than 100 µS cm^{-1} could indicate that environments were impacted. In Lake Furnas, the measurements were well below this limit, indicating the region may not present high pollution loads.

In addition to these physical-chemical characteristics, turbidity of the water samples was also measured. According to the CONAMA Resolution 357/2005[32], turbidity values for a class 2 river should stay below 100 Nephelometric Turbidity Unit (NTU). Values above this limit may indicate a constant load of pollutants within the area. The measured values were lower than 6.0 NTU, indicating the sample sites have reduced turbidity.

Table 5. Maximum and minimum values of pH, dissolved oxygen, temperature, and conductivity measured in the surroundings of collection sites.

Sampling date	pH	Dissolved oxygen / mg L^{-1}	Temperature / °C	Conductivity / µS cm^{-1}
09-03-2016	6.16 – 7.11	5.07 – 5.65	27.96 – 28.31	20 – 27
07-04-2016	7.61 – 8.36	6.99 – 8.14	28.23 – 29.21	30 – 33
28-04-2016	6.42 – 6.77	3.44 – 4.60	26.63 – 27.03	14 – 22
19-05-2016	6.29 – 6.44	2.77 – 3.75	23.98 – 24.25	28 – 39

3.3.2 SPE and chromatographic analysis of samples collected at Furnas Lake

The SPE of water samples were carried out at a flow rate of 3 mL min^{-1}, proceeding was as follows: adjusting the pH of the sample to 8, conditioning the cartridge with 5.00 mL of methanol and passing 1 L of the sample through the cartridge. After percolation of the sample, the cartridge was washed with 1.00 mL of wash solution (water: 5% methanol) and the analytes were eluted with 5.00 mL of methanol: ethyl acetate to the cartridge. Subsequently, the eluates were dried in a water bath at 70 °C using a heating mantle, resuspended in 1.00 mL of acetonitrile, placed in an ultrasonic bath for 3 min, filtered with 0.45 µm pore-size filters and injected into the HPLC system. The fluorescence detector was more sensitive, had lower detection and quantification limits, and recovery values closer to 100% when compared to the validated methodology. Therefore, it was decided to analyze all estrogens, except estrone, which was detected only by DAD using the validated method.

It can be observed that the analytes presented good separation and the retention times obtained are close to the values obtained in the validation of the analytical methodology employed. It is worth mentioning that estrone was not found in any of the samples collected, therefore, no chromatogram showing this estrogen was obtained.

Table 6. Mean concentration and standard deviation found for estriol, 17β-estradiol and 17α-ethynylestradiol in the water samples collected in the study region.

Sampling / date	Estrogen	Sampling site average recovery / ng L^{-1} ± standard deviation				
		1	2	3	4	5
1 (12/07/2015)	E3	ND	ND	ND	202 ± 9	237 ± 11
	E2	76 ±1	75 ±1	101 ± 1	*1732 ± 9	*2089 ± 7
	EE2	ND	ND	ND	322 ± 10	320 ± 11
2 (03/09/2016)	E3	ND	< LOQ	157 ± 5	366 ± 5	181 ± 14
	E2	154 ± 4	116 ± 1	124 ± 1	*9998 ± 3	195 ± 3
	EE2	ND	ND	118 ± 1	209 ± 5	196 ± 4
3 (04/07/2016)	E3	318 ± 3	208 ± 7	182 ± 1	120 ± 1	110 ± 3
	E2	*2536 ± 1	*1993 ± 1	*1792 ± 1	*1415 ± 1	*1066 ± 1
	EE2	422 ± 5	284 ± 3	256 ± 10	180 ± 2	141 ± 1
4 (04/28/2016)	E3	153± 18	ND	ND	ND	ND
	E2	*1101 ±0.0	411 ± 7	436 ± 2	236 ±1	231 ± 1
	EE2	143 ± 4	67 ± 3	63 ± 2	< LOQ	< LOQ
5 (05/19/2016)	E3	ND	ND	ND	ND	ND
	E2	321 ± 2	183 ± 3	254 ± 1	194 ± 1	206 ± 1
	EE2	76 ±0.0	< LOQ	< LOQ	< LOQ	< LOQ

*Samples that were diluted 10 times to understand the concentration range of the analytical curve of the validated method; ND = Not Detected.

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The results presented in Tab. 6 refer to the average concentrations for each estrogen found in the five collection points during the five sampling campaigns conducted between December 2015 and May 2016.

In general, the results in Tab. 6 show that each water sample analyzed was positive for at least one of the tested estrogens. 17β-estradiol (E2) was detected and quantified in all samples, while Estriol (E3) and 17α-ethynylestradiol (EE2) were detected in 48 and 80% of the samples, respectively. The maximum concentrations determined were: E3 366 ± 5; E2 9998 ± 3; and EE2 422 ± 5 ng L^{-1}. Estrone (E1) was neither detected nor quantified by the employed optimized analytical methodology (Fig. 3). However, since E2 is oxidized to E1 after excretion and because of the high E2 concentration found, E1 might also be present in Furnas Lake. On the other hand, due to the sorption coefficient value of estrone (4882), the highest among these four estrogens, a high sorption potential can be expected in soils and sediments, which would reduce their concentration within the water column[36-38].

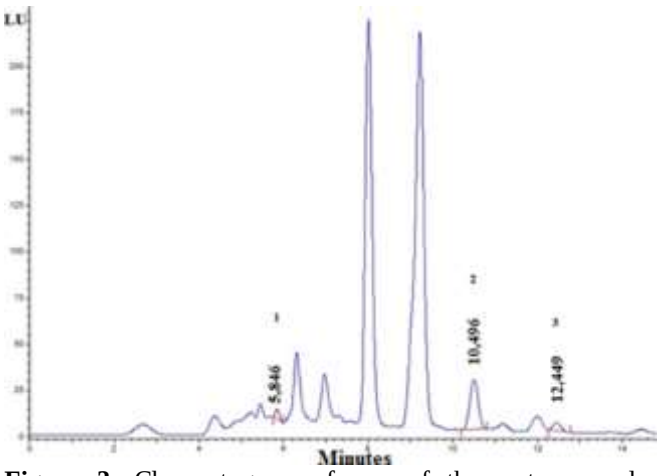

Figure 3. Chromatogram of one of the water samples collected in Lake Furnas concerning the separation of estrogens with FLD detection: 1) estriol; 2) 17β-estradiol; 3) 17α-ethynylestradiol.

One possible explanation for the absence of estrogens in some of the analyzed water samples would be their presence in the conjugated form, basically in the form of sulfates and glucuronides. These polar conjugates are biologically inactive and more soluble in water when compared to their corresponding unconjugated counterparts. Therefore, to monitor the presence of these estrogens in this form, a specific analytical method would be required.

Similar results to these presented in the current study were reported by Montagner and Jardim[39]. An analytical method was optimized for determination of 15 emerging contaminants within surface waters from the Atibaia River, in the city of Campinas (SP - Brazil), using solid phase extraction and high-performance liquid chromatography with ultraviolet detection with diode array and fluorescence. The method was applied for spatial and seasonal monitoring of these emerging contaminants, which included estrone (E1), 17β-estradiol (E2) and 17α-ethynylestradiol (EE2). Among these, E2 was the most frequently detected, present in 35% of the 26 samples and found in high concentrations, which ranged from 106-6806 ng L^{-1}. On the other hand, EE2 was found in only three samples and E1 was not detected.

Many studies in the literature report the determination of these estrogens; however, they were detected in lower concentrations when compared to those in this study. Monteiro and Andrade[40] developed and validated an analytical methodology for determination of the synthetic estrogens levonorgestrel (LNG) and 17α-ethynylestradiol (EE2) in surface waters of the Sapucaí River, in Itajubá (MG - Brazil). Samplings were carried out within the urban region of the river during the rainy season. EE2 was found in the 5 sampling sites at concentrations ranging from 67.4-99.1 ng L^{-1}, whereas LNG was not found in any sampling site. In Belo Horizonte (MG, Brazil), Moreira *et al.*[41] analyzed water samples from the river called Rio das Velhas, Morro Redondo and Vargem das Flores lake, and determined concentrations between 1.5-36.8 ng L^{-1} for 17β-estradiol and 3.0-54 ng L^{-1} for 17α-ethynylestradiol within these samples. Other authors reported lower concentrations of E2 (0.3 and 1.3 ng L^{-1}) and EE2 (0.5 and 3, 0 ng L^{-1}) form groundwater samples in Rhône-Alpes region (France)[42]. In Austria, Bursch and colleagues[43] evaluated 27 surface water and 59 groundwater samples, totaling 261 and 112, respectively. In more than 50% of surface water samples, E2 and E1 were detected at low concentrations (average of 0.13 and 0.35 ng L^{-1}, respectively). While in groundwater samples, E2 was detected in 50% of samples with a maximum value of 0.79 ng L^{-1}.

In contrast, some studies in Brazil and in other countries found these estrogens in concentrations even higher than the reported here. In analysis of surface water samples from the city of Campinas (SP - Brazil), the identified concentrations of E1, E2, and EE2 were in μg L^{-1} scales, ranging from 3.5-5.0, 1.9-6.0 and 1.2-3.5, respectively[44]. Daniel and Lima[45] tested for the presence of estrogen, estriol, 17β-estradiol, and 17α-ethynylestradiol in water samples from the Tubarão River, in the border of municipalities of Santo André and Mauá (SP - Brazil). In this study, significant

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concentrations of estriol (0.4 g L^{-1}) and estrone (0.32 g L^{-1}) were detected. Chaves[46] validated an analytical method and used SPE and HPLC-UV for determination of endocrine disruptors BFA, E2, EE2 and E1 in the Paraíba do Sul River, Aparecida (SP - Brazil). In the work, surface water samples were taken at different depths, close to the water collection point for public supply, which is located 50 m downstream from the sewage disposal site of the National Sanctuary of Aparecida. High concentrations were found for all analytes, ranging from 0.032-0.088 μg L^{-1} for BFA, 0.009-0.40 μg L^{-1} for E2, 0.07-0.6 μg L^{-1} for EE2 and 0.107-0.91 μg L^{-1} for E1. The author inferred that the values obtained may be associated with the number of visitors in the city, the inefficiency of conventional sewage treatment system to remove such contaminants, rainfall index and temperature. Since these endocrine disruptors may represent risks to biota at concentrations of as low as ng L^{-1}, such exposures at μg L^{-1} and g L^{-1} scales, are even more alarming.

The works described above confirm that the results found for Furnas Lake in Alfenas is not a particular case and show that rivers from different parts of Brazil are contaminated with endocrine disruptors.

4. Conclusions

The validated analytical methodology for monitoring estrone, estriol, 17β-estradiol and 17α-ethynylestradiol was adequate, according to the evaluated figures of merit. In view of the responses obtained with the fluorescence detector (FLD), such as high sensitivity, optimal values of recovery and low LOQ and LOD, it was decided to work with this detector for all estrogens, except estrone, which was only detected by DAD.

The use of chemometric methods for experimental design and data processing was efficient. Considering this and the analysis of response surface plots, it was possible to determine the best performing experiment to extract the four studied estrogens.

The five sampling campaigns were carried out from December 2015 to May 2016 in 5 different sites. In general, all estrogens were found in all samples of at least one of these collection points, only E1 was not. Concentrations of E3, E2 and EE2 ranged from 11-366, 75-9998, and 63-422 ng L^{-1}, respectively.

In view of the results obtained, it would be interesting to deepen further studies on this topic and to develop and validate analytical methodologies for estrogen determination in their conjugated forms. Additionally, other emerging contaminants could also be analyzed within this same studied region as in other parts of Furnas Lake, since this subject has been neglected by the scientific community until the present moment.

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References

[1] United Nations Educational, Scientific and Cultural Organization (UNESCO). Water for a sustainable world. 2015. http://unesdoc.unesco.org/images/0023/002318/231823E.pdf .

[2] Grassi, M. T. As águas do planeta Terra, Cadernos Temáticos de Química Nova na Escola 1 (1) (2001) 31-40. http://qnesc.sbq.org.br/online/cadernos/01/aguas.pdf.

[3] Avigliano, E., Schenone, N. F., Human health risk assessment and environmental distribution of trace elements, glyphosate, fecal coliform and total coliform in Atlantic Rainforest mountain rivers (South America), Microchemical Journal 122 (2015) 149-158. https://doi.org/10.1016/j.microc.2015.05.004.

[4] United States Census Bureau (USCB), International Data Base (IDB). U.S. and World Population Clock Census Bureau, Washington, 2015. http://www.census.gov/popclock/.

[5] Silva, C. G. A., Collins, C. H., Aplicações de cromatografia líquida de alta eficiência para o estudo de poluentes orgânicos emergentes, Química Nova 34 (4) (2011) 665-676. https://doi.org/10.1590/S0100-40422011000400020.

[6] Deblonde, T., Cossu-Leguille, C., Hartemann, P., Emerging pollutants in wastewater: A review of the literature, International Journal of Hygiene and Environmental Health 214 (6) (2011) 442-448. https://doi.org/10.1016/j.ijheh.2011.08.002.

[7] Jurado, A., Vàzquez-Suñé, E., Carrera, J., Alda, M. L., Pujades, E., Barceló, D., Emerging organic contaminants in groundwater in Spain: A review of sources, recent occurrence and fate in a European context, Science of The Total Environment 440 (2012) 82-94. https://doi.org/10.1016/j.scitotenv.2012.08.029.

[8] Luo, Y., Guoa, W, Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., Liang, S., Wang, X. C., A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment,

Science of The Total Environment 473-474 (2014) 619-641. https://doi.org/10.1016/j.scitotenv.2013.12.065.

[9] Braz, F. S., Silva, M. R. A., Silva, F. S., Andrade, S. J., Fonseca, A. L., Kondo, M. M., Photocatalytic Degradation of Ibuprofen Using TiO_2 and Ecotoxicological Assessment of Degradation Intermediates against *Daphnia similis*, Journal of Environmental Protection 5 (7) (2014) 620-626. https://doi.org/10.4236/jep.2014.57063.

[10] Candido, J. P., Andrade, S. J., Fonseca, A. L., Silva, F. S., Silva, M. R. A., Kondo, M. M., Ibuprofen removal by heterogeneous photocatalysis and ecotoxicological evaluation of the treated solutions, Environmental Science and Pollution Research 23 (2016) 19911-19920. https://doi.org/10.1007/s11356-016-6947-z.

[11] Barreiros, L., Queiroz, J. F., Magalhães, L. M., Silva, A. M. T., Segundo, M. A., Analysis of 17-*β*-estradiol and 17-*α*-ethinylestradiol in biological and environmental matrices — A review, Microchemical Journal 126 (2016) 243-262. https://doi.org/10.1016/j.microc.2015.12.003.

[12] Mei, S., Wu, D., Jiang, M., Lu, B., Lim, J.-M., Zhou, Y.-K., Lee, Y.-I., Determination of trace bisphenol A in complex samples using selective molecularly imprinted solid-phase extraction coupled with capillary electrophoresis, Microchemical Journal 98 (1) (2011) 150-155. https://doi.org/10.1016/j.microc.2011.01.003.

[13] Giulivo, M., Alda, M. L., Capri, E., Barceló, D., Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review, Environmental Research 151 (2016) 251-264. https://doi.org/10.1016/j.envres.2016.07.011.

[14] Diamanti-Kandarakis, E., Bourguignon, J.-P., Giudice, L. C., Hauser, R., Prins, G. S., Soto, A. M., Zoeller, R. T., Gore, A. C., Endocrine-disrupting chemicals: an endocrine society scientific statement, Endocrine Reviews 30 (4) (2009) 293-342. https://doi.org/10.1210/er.2009-0002.

[15] Nohynek, G. J., Borgert, C. J., Dietrich, D., Rozman, K. K. Endocrine disruption: fact or urban legend? Toxicology Letters 223 (3) (2013) 295-305. https://doi.org/10.1016/j.toxlet.2013.10.022.

[16] Leonard, J. A., Cope, W. G., Hammer, E. J., Barnhart, M. C., Bringolf, R. B., Extending the toxicity-testing paradigm for freshwater mussels: Assessing chronic reproductive effects of the synthetic estrogen 17α-ethynylestradiol on the unionid mussel *Elliptio complanata*, Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 191 (2017) 14-25. https://doi.org/10.1016/j.cbpc.2016.09.002.

[17] Omar, T. F. T., Ahmad, A., Aris, A. Z., Yusoff, F. M. Endocrine disrupting compounds (EDCs) in environmental matrices: Review of analytical strategies for pharmaceuticals, estrogenic hormones, and alkylphenol compounds, TrAC Trends in Analytical Chemistry 85 (Part C) (2016) 241-259. https://doi.org/10.1016/j.trac.2016.08.004.

[18] Instituto Brasileiro de Geografia e Estatística (IBGE). Censo demográfico de 2009. http://censo2010.ibge.gov.br/.

[19] Instituto Mineiro de Gestão das Águas (IGAM). Plano Diretor de Recursos Hídricos da Bacia Hidrográfica do Entorno do Lago de Furnas, Alfenas. http://www.igam.mg.gov.br/gestao-das-aguas/plano-de-recursos-hidricos.

[20] Amarante Junior, O. P., Caldas, E. P. A., Brito, N. M., Santos, T. C. R., Vale, M. L. B. F., Validação de métodos analíticos: uma breve revisão, Cadernos de Pesquisa. 12 (1-2) (2001) 116-131. https://docplayer.com.br/39827741-Validacao-de-metodos-analiticos-uma-breve-revisao.html.

[21] Agência Nacional de Vigilância Sanitária (ANVISA). Resolução n° 899, de 29 de maio de 2003. http://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2003/res0899_29_05_2003.html.

[22] Baccan, N., Andrade, J. C., Godinho, O. C. S., Barone, J. S. Química Analítica Quantitativa Elementar. Edgard Blucher, São Paulo, 3rd ed., 2001.

[23] Amarante Junior, O. P., Polese, L., Santos, T. C. R., Ribeiro, M. L. Avaliação da exatidão e da precisão de métodos de análise de resíduos de pesticidas mediante ensaios de recuperação, Pesticidas: Revista de Ecotoxicologia e Meio Ambiente 12 (2002) 155-168. https://doi.org/10.5380/pes.v12i0.3157.

[24] Brito, N. M., Amarante Junior, O. P., Polese, L., Ribeiro, M. L., Validação de métodos analíticos: estratégia e discussão, Pesticidas: Revista de Ecotoxicologia e Meio Ambiente 13 (2003) 129-146. https://doi.org/10.5380/pes.v13i0.3173.

[25] Instituto Nacional de Metrologia, Normalização e Qualidade Industrial (INMETRO). Orientações sobre Validação de Métodos de Ensaios Químicos DOQ-CGCRE-008, 2010. http://www.inmetro.gov.br/Sidoq/Arquivos/Cgcre/DOQ/DOQ-Cgcre-8_05.pdf.

[26] Ribani, M., Bottoli, C. B. G., Collins, C. H., Jardim, I. C. S. F., Melo, L. F. C., Validação em métodos cromatográficos e eletroforéticos, Química Nova 27 (5) (2004) 771-780. https://doi.org/10.1590/S0100-40422004000500017.

[27] Ribani, M., Collins, C. H., Bottoli, C. B. G., Desenvolvimento e validação de método para separação de isoflavonas em extrato seco de soja, Ciência e Natura 36 (3) (2014) 501-510. https://doi.org/10.5902/2179460X12817.

[28] Companhia Ambiental do Estado de São Paulo (CETESB), Agência Nacional de Água (ANA). Guia Nacional de Coleta e Preservação de Amostras: água, sedimentos, comunidades aquáticas e efluentes líquidos. CETESB, ANA, Brasília, 2011. https://arquivos.ana.gov.br/institucional/sge/CEDOC/Catalogo/2012/GuiaNacionalDeColeta.pdf.

[29] Agência Nacional de Água (ANA). Boletim de Monitoramento do Reservatório de Furnas. Brasília, 3 (12) (2015) 1-13. https://arquivos.ana.gov.br/saladesituacao/BoletinsMensais/Furnas/Boletim_Monitoramento_Furnas_2015_12.pdf.

[30] PRADO, P. S.; SOUZA, C. C.; BAZZOLI, N.; RIZZO, E. Reproductive disruption in lambari Astyanax fasciatus from a Southeastern Brazilian reservoir. Ecotoxicology and Environmental Safety 74 (2011), 1879–1887. https://doi.org/10.1016/j.ecoenv.2011.07.017.

[31] GOOGLE EARTH, 2020. https://www.google.com.br/intl/pt-BR/earth/.

[32] Brasil. Conselho Nacional do Meio Ambiente (CONAMA). Resolução n. 357 de 17 de março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. 2005. http://www2.mma.gov.br/port/conama/legiabre.cfm?codlegi=459.

[33] G1-Sul de Minas. Falta de sol pode ter contribuído para morte de peixes no Lago de Furnas. 2016. http://g1.globo.com/mg/sul-de-minas/noticia/2016/05/falta-de-sol-pode-ter-contribuido-para-morte-de-peixes-no-lago-de-furnas.html.

[34] Globo Rural. Mortandade de peixes no Lago de Furnas (MG) prejudica criadores. 2013. http://globoplay.globo.com/v/2451854/.

[35] Companhia de Tecnologia de Saneamento Ambiental (CETESB). Significado ambiental e sanitário das variáveis de qualidade das águas e dos sedimentos e metodologias analíticas e de amostragem. Série de relatórios: Qualidade das águas interiores no estado de São Paulo. p.43, 2009.

[36] Dolar, D., Gros, M., Rodriguez-Mozaz, S., Moreno, J., Comas, J., Rodriguez-Roda, I., Barceló, D., Removal of emerging contaminants from municipal wastewater with an integrated membrane system, MBR-RO, Journal of Hazardous Materials 239-240 (2012) 64-69. https://doi.org/10.1016/j.jhazmat.2012.03.029.

[37] Guedes-Alonso, R., Montesdeoca-Esponda, S., Sosa-Ferreira, Z., Santana-Rodríguez, J. J., Liquid chromatography methodologies for the determination of steroid hormones in aquatic environmental systems, Trends in Environmental Analytical Chemistry 3-4 (2014) 14-27. https://doi.org/10.1016/j.teac.2014.10.001.

[38] Hamid, H., Eskicioglu, C., Fate of estrogenic hormones in wastewater and sludge treatment: A review of properties and analytical detection techniques in sludge matrix, Water Research 46 (18) (2012) 5813-5833. https://doi.org/10.1016/j.watres.2012.08.002.

[39] Montagner, C. C., Jardim, W. F. Spatial and seasonal variations of pharmaceuticals and endocrine disruptors in the Atibaia River, São Paulo State (Brazil), Journal of the Brazilian Chemical Society 22 (8) (2011) 1452-1462. https://doi.org/10.1590/S0103-50532011000800008.

[40] Monteiro, B. R., Andrade, S. J., Desenvolvimento e validação de metodologia analítica para determi-nação dos hormônios sintéticos 17α-etinilestradiol e levonorgestrel em águas superficiais, Ciência e Natura 37 (2) (2015) 391-400. https://doi.org/10.5902/2179460X17320.

[41] Moreira, D. S., Aquino, S. F., Afonso, R. J. C. F., Santos, E. P. P. C., Pádua, V. L. Occurrence of endocrine disrupting compounds in water sources of Belo Horizonte Metropolitan Area, Brazil. Environmental Technology 30 (10) (2009) 1041-1049. https://doi.org/10.1080/09593330903052830.

[42] Vulliet, E., Wiest, L., Baudot, R., Grenier-Loustalot, M.-F., Multi-residue analysis of steroids at sub-ng/L levels in surface and ground-waters using liquid chromatography coupled to tandem mass spectrometry, Journal of Chromatography A 1210 (1) (2008) 84-91. https://doi.org/10.1016/j.chroma.2008.09.034.

[43] Bursch, W., Fuerhacker, M., Gemeiner, M., Grillitsch, B., Jungbauer, A., Kreuzinger, N., Moestl, E., Scharf, S., Schmid, E., Skutan, S., Walter, I., Endocrine disrupters in the aquatic environment: the Austrian approach - ARCEM, Water Science & Technology 50 (5) (2004) 293-300. https://doi.org/10.2166/wst.2004.0340.

[44] Ghiselli, G., Jardim, W. F., Interferentes endócrinos no ambiente, Química Nova 30 (3) (2007) 695-706. https://doi.org/10.1590/S0100-40422007000300032.

[45] Daniel, M. S., Lima, E. C., Determinação simultânea de estriol, β-estradiol, 17α-etinilestradiol e estrona empregando-se extração em fase sólida (SPE) e cromatografia líquida de alta eficiência (HPLC). Revista Ambiente & Água 9 (4) (2014) 688-695. https://doi.org/10.4136/ambi-agua.1346.

[46] Chaves, K. S., Determinação dos desreguladores endócrinos bisfenol-A, β-estradiol, 17α-etinilestradiol e estrona no Rio Paraíba do Sul. 133 p. Dissertação de mestrado – Escola de Engenharia de Lorena, Universidade de São Paulo, Lorena, 2015. https://www.teses.usp.br/teses/disponiveis/97/97131/tde-24032016-090046/publico/BID15010_C.pdf.

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Comparison between two Polyethersulfone concentrations in hollow fiber ultrafiltration membranes. Is it worth to use more polymer?

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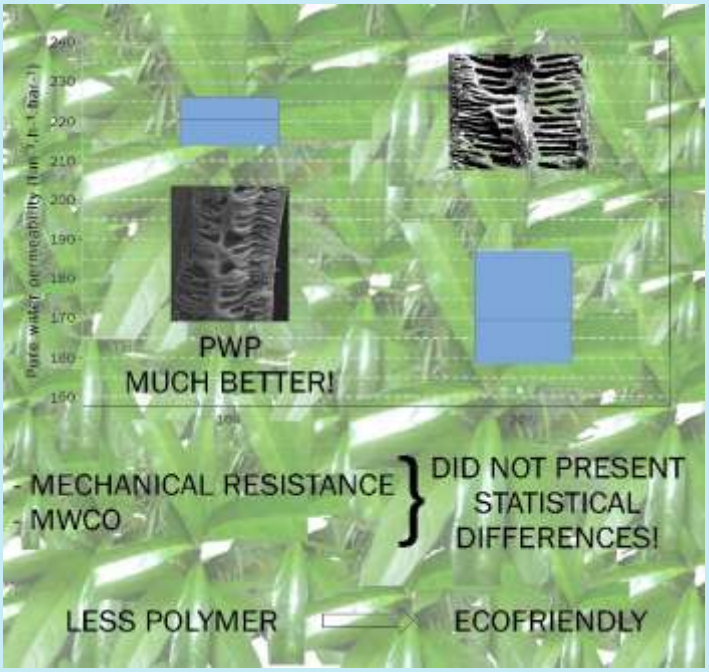
ABSTRACT: Polyethersulfone (PES) hollow fiber membranes were fabricated using dry-jet wet spinning technique, a phase inversion method, with 16 and 20% PES, N-methyl-2-pyrrolidone (NMP) as solvent and tap water as nonsolvent, in order to evaluate if the amount of polymer has a significant effect on its properties. They were characterized using SEM for a morphological analysis, a continuous system to measure pure water permeability (PWP) and molecular weight cutoff (MWCO), and a universal testing machine to tensile tests. The obtained results for PWP was an average of about 220 L m^{-2} h^{-1} bar^{-1} for the 16% PES membrane and 174 L m^{-2} h^{-1} bar^{-1} for the 20% PES membrane. The results of mechanical resistance and MWCO did not present statistical differences. Thus, it is confirmed that the 16% PES membrane can be as good as the 20%, despite using less polymer, a finding that can further motivate membrane modification studies and other related works.



1. Introduction

Polymeric hollow fiber membranes were first developed by Dow Chemical in 1966 and since then, due to their properties as high processability and low cost, they are the most common material used for membrane fabrication[1–3]. Hollow fiber membranes are used in several areas that demand a separation process, such as pharmaceutical industry, food industry, water and wastewater treatment plants and the petroleum sector[4,5].

The method of phase inversion is one of the most important techniques of membrane fabrication[6] for hollow fibers. It is based on an extrusion of a polymer solution through a spinneret, which will return to the solid state[6,7]. Dry-jet wet spinning follows this idea with a phase inversion beginning with an induced evaporation that occurs in the air gap and finishing in the coagulation bath with a phase inversion induced by diffusion[1].

Polyethersulfone (PES) has been widely used because it can tolerate a large range of pH, has a good thermal stability and excellent chemical and mechanical resistance[6,8,9]. This polymer and the conditions for the solution spinning determine the morphology and properties of hollow fiber membrane, such as selectivity[7,10].

Despite numerous studies related to hollow fiber membranes composed of PES[11–16] and the known advantages of membrane separation processes[17–19], there is a worldwide concern with the amount of microplastics that are emerging around the globe[20–23] and not only with energy efficiency, pollutant emissions and other sustainability issues. Lower consumption of plastic is a policy growing in many countries, seeking to encourage consumers to find a way to reduce it on a daily basis[24].

Based on this tendency, the present work focuses on studying the properties of hollow fiber membranes made of 16 and 20% PES, evaluating if there are significant differences among the results obtained that justify the use of a bigger quantity of polymer. Future studies of membrane modification can be based on compositions using smaller amounts of polymer without impairing its application, demonstrating its importance.

2. Experimental

2.1 Materials

Polyethersulfone (VERADEL 3000P with MW = 63,000 g Mol-1) from Solvay Advanced Polymer was dried for 4 h in an oven at 100 °C before utilizing it for fabrication of the dope solution. N-methyl-2-pyrrolidone (NMP) with purity >99% from Labsynth Produtos para Laboratórios Ltda. was used as received as the solvent for the polymer. Tap water was used as the nonsolvent agent, as bore fluid and in coagulation bath.

2.2 Solution preparation

The dope solution was prepared with 16 and 20% (weight/weight) concentration of PES, with the remaining concentration (84 and 80%, respectively) being of NMP. The polymer was slowly added to the solvent, taking 1 h for complete addition, at room temperature. Then, the solution remained for about 18 h at 200 r.p.m. stirring, assuring complete solubilization of the polymer and homogenization, to be finally degassed in an ultrasonic bath for 1 h, eliminating any bubbles of air trapped into solution.

2.3 Hollow fiber production

The solution was spinning with a solution flow rate of 2 mL min^{-1} and a bore fluid flow rate of 4 mL min^{-1}, using dry-jet wet spinning method with an air gap of 2 cm and take-up speed of 4 mL min^{-1}, at room temperature. A schematic representation of the spinneret and fabricating hollow fiber membrane processes is shown in Fig. 1.

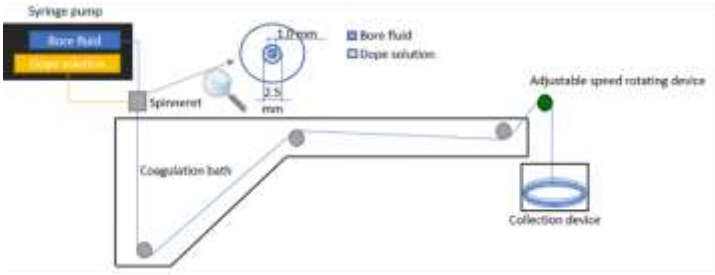


Figure 1. Schematic representation of fabricating hollow fiber membrane system.

Hollow fiber membranes were stored in demineralized water to keep their integrity. To realize some characterization procedures, these were dried following the steps described elsewhere[25].

2.4 Membrane characterization

The produced membranes were investigated about its morphology, permeability, mechanical resistance and MWCO (molecular weight cutoff). The data

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obtained were treated to remove outliers and statistical analysis of the results was done using Minitab® 18.1, from Minitab, Inc., assuming a significance level of 0.05 and equality of variances.

2.4.1 Morphological analysis

The cross-sectional of the hollow fiber membranes was observed by a scanning electron microscope (Zeiss SEM model EVO MA10) with an acceleration voltage of 20 kV. For this, dried membranes were cut under liquid nitrogen and sputter-coated with a thin film of gold-platinum. These procedures under nitrogen are necessary to produce a clean and brittle fracture and the coating is required due to the nature of polymeric material, not electrically conductive. Doing this way, it is possible to see the microstructure of the produced membranes.

2.4.2 Pure water permeability

Pure water permeability (PWP) measurements were estimated in an experimental setup, where a module made from low-density polyethylene (LDPE) was utilized with two hollow fiber membranes with 30 cm effective lengths, folded in half and fed topside in an inside out mode.

A syringe pump with a constant flow rate of 0.5 mL min^{-1} supplied demineralized water to the module and the water production measurement was started after thirty minutes to achieve steady-state conditions. Records were made every five minutes for one hour, similar to the procedure previously described[26]. The permeation flux (J_w) through the membrane was calculated following the Eq. 1:

$$J_w = \frac{V}{A*t*\Delta P} \quad (1)$$

where: J_w = Water permeability (L m^{-2} h^{-1} bar^{-1}); V = Volume of permeate (L); A = Inner surface area (m^{-2}); t = Time (h); ΔP = Transmembrane pressure (bar).

2.4.3 Mechanical resistance

The mechanical resistance of the hollow fiber membranes was investigated by means of tensile tests, measured using an Instron universal testing system and a 100 N load cell and constant rate of 1 mm s^{-1}, with an initial gauge length of 30 mm. The test method was based on ASTM 1557-14 Standard Test Method for Tensile Strength and Young's Modulus of Fibers.

Five dried samples with 100 mm length were tested for each experimental result, determining membrane tensile strength, elongation at break and Young's modulus.

2.4.4 Molecular weight cutoff (MWCO)

The MWCO was investigated using 200 ppm polyethylene glycol (PEG) solution with molecular weights of 10 kDa, 32 kDa, 90 kDa and 150 kDa, into the same system and module described in 2.4.2.

The concentration of PEG in permeate and in retentate was analyzed by a UV-VIS spectrophotometer (WUV-M51, Weblaborsp) at a wavelength of 254 nm and the MWCO was determined from the rejection of PEG solution, following Eq. 2:

$$\boldsymbol{R = \left(1 - \frac{C_p}{C_r}\right) * 100\%} \quad (2)$$

where: R = Rejection (%); C_p = Permeate concentration (Da); C_r = Retentate concentration (Da).

3. Results and discussion

3.1 Morphological analysis

To evaluate the impact of polymer concentration on the morphology of hollow fiber membranes, SEM was used to observe its enlarged cross section and outside face of the samples. Figures 2 and 3 show both membranes resulted in an outside dense skin and a double layered finger-like structure pore, but it is possible to see that the 16% PES concentration membrane has a bigger aperture at the end of the pores, nearby their center.

Double layer finger-like and dense skin were expected microstructure characteristics for these membranes. They occur because water is a strong nonsolvent for the polymer, that provides a fast coagulation, and this formation is a consequence of it, like reported previously[11,27,28].

The morphological analysis indicates that the permeation flux tends to be better in the 16% PES concentration membrane. This tendency is verified by pure water permeability investigation results, but it can happen because its pores had an opening larger than the other membrane, facilitating the flow of the fluid through it.

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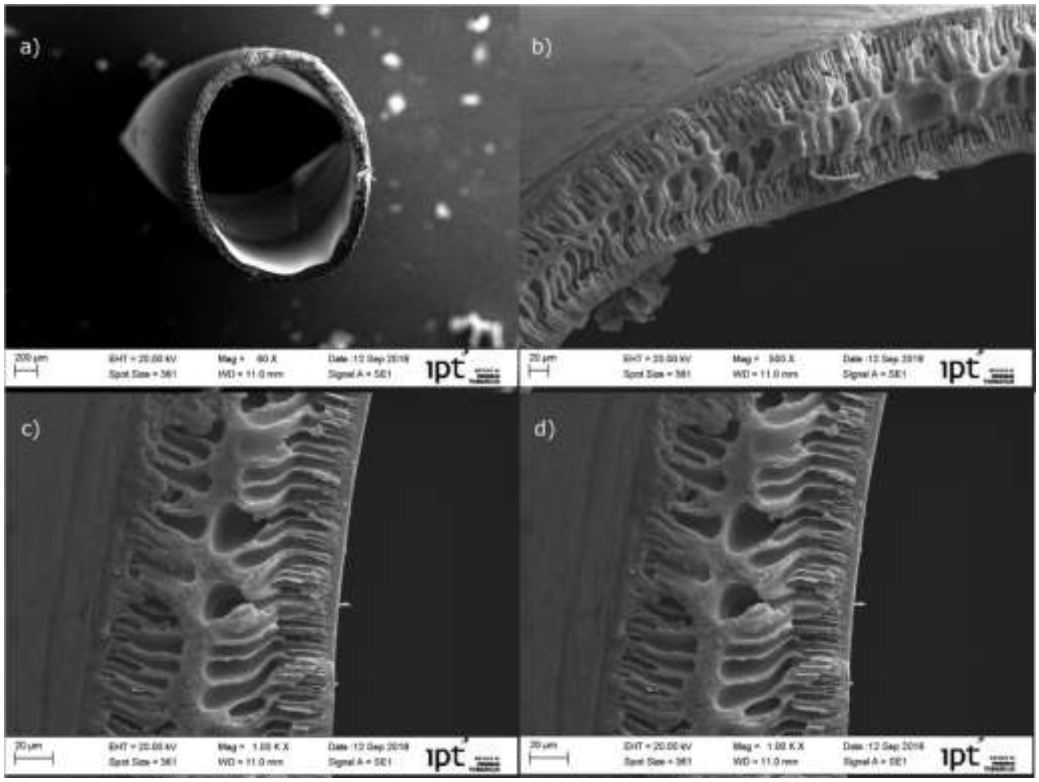

Figure 2. SEM images of 16% PES sample: a) full cross section of the hollow fiber membrane, b) and c) cross section of the hollow fiber membrane with different magnifications, and d) outer skin.

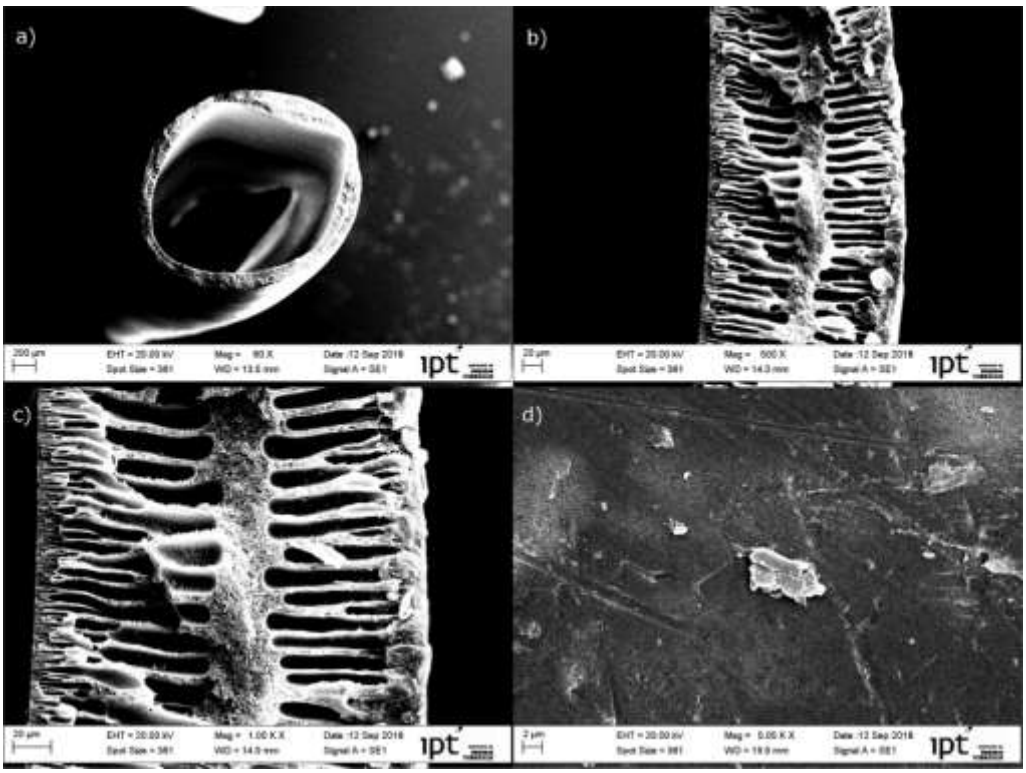

Figure 3. SEM images of 20% PES sample: a) full cross section of the hollow fiber membrane, b) and c) cross section of the hollow fiber membrane with different magnifications, and d) outer skin.

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3.2 Pure water permeability

The results of demineralized water permeation flux for the hollow fiber membranes produced are shown in Fig. 4. It shows an average permeability about 220 L m^{-2} h^{-1} bar^{-1} for the 16% PES concentration membrane and 174 L m^{-2} h^{-1} bar^{-1} for the 20% PES concentration membrane. These results agree with the SEM analysis showed above and also with reports in the literature[29,30].

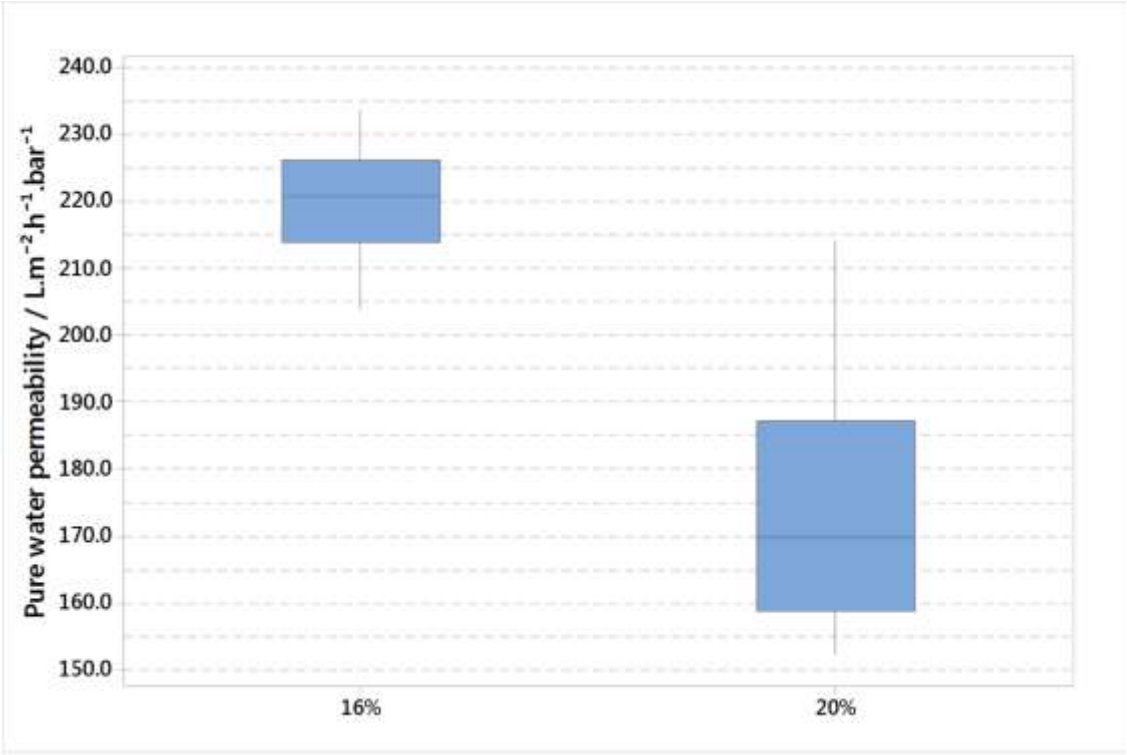

Figure 4. Pure water permeability of 16% PES and 20% PES membranes.

Table 1 shows statistical analysis of the results of permeability presented. According to ANOVA evaluation, there is a statistical difference between them.

Table 1. ANOVA statistical analysis of membranes PWP.

Source	DF	Adj. SS	Adj. MS	F-Value	P-Value
Factor	1	12208	12207.90	56.11	0.000
Error	22	4787	217.60		
Total	23	16995			

3.3 Mechanical resistance

The results of the mechanical properties investigation for membranes are summarized in Tab. 2.

Table 2. Mechanical resistance properties of membranes.

Sample	Tensile strength / MPa		Elongation at break / %		Young's Modulus / MPa	
	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
16%	220	9	15	2	180	47
20%	174	19	15	2	279	54

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These results show there is a small variance between the average of tensile strength, elongation at break and Young's modulus. With the analysis of results, it is possible to note there is no relevant difference between the 16 and 20% PES membranes (Tabs. 3 to 5).

Table 3. ANOVA statistical analysis of membranes tensile strength.

Source	DF	Adj. SS	Adj. MS	F-Value	P-Value
Factor	1	0.0918	0.09177	0.02	0.884
Error	7	28.0531	4.00759		
Total	8	28.1449			

Table 4. ANOVA statistical analysis of membranes elongation at break.

Source	DF	Adj. SS	Adj. MS	F-Value	P-Value
Factor	1	167.6	167.6	1.18	0.339
Error	4	568.8	142.2		
Total	5	736.4			

Table 5. ANOVA statistical analysis of membranes Young's Modulus.

Source	DF	Adj. SS	Adj. MS	F-Value	P-Value
Factor	1	14656	14656	5.73	0.075
Error	4	10235	2559		
Total	5	24891			

3.4 MWCO

The data obtained for MWCO were recorded up to PEG 150 kDa MW and the membranes were not able to remove 90% of this substance, which is the concept of MWCO. Thus, to estimate this important characteristic of membranes and knowing that the typical rejection curve is sigmoidal[31,32], an extrapolation was made with the results (Fig. 5 and 6).

Based on the point that represents 90% of removal in these curves, values of 271 kDa and 279 kDa are the MWCO estimated of 16% PES membrane and 20% PES membrane, respectively. The statistical analysis does not show a relevant difference between both results, as demonstrated in Tab. 6.

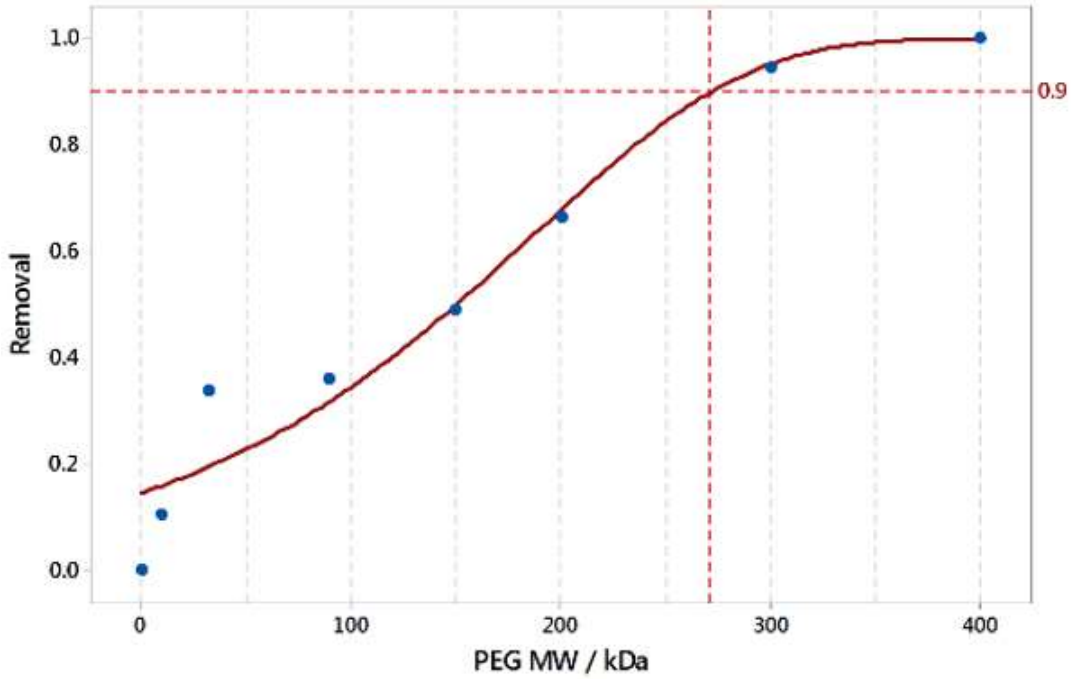

Figure 5. 16% PES membrane MWCO.

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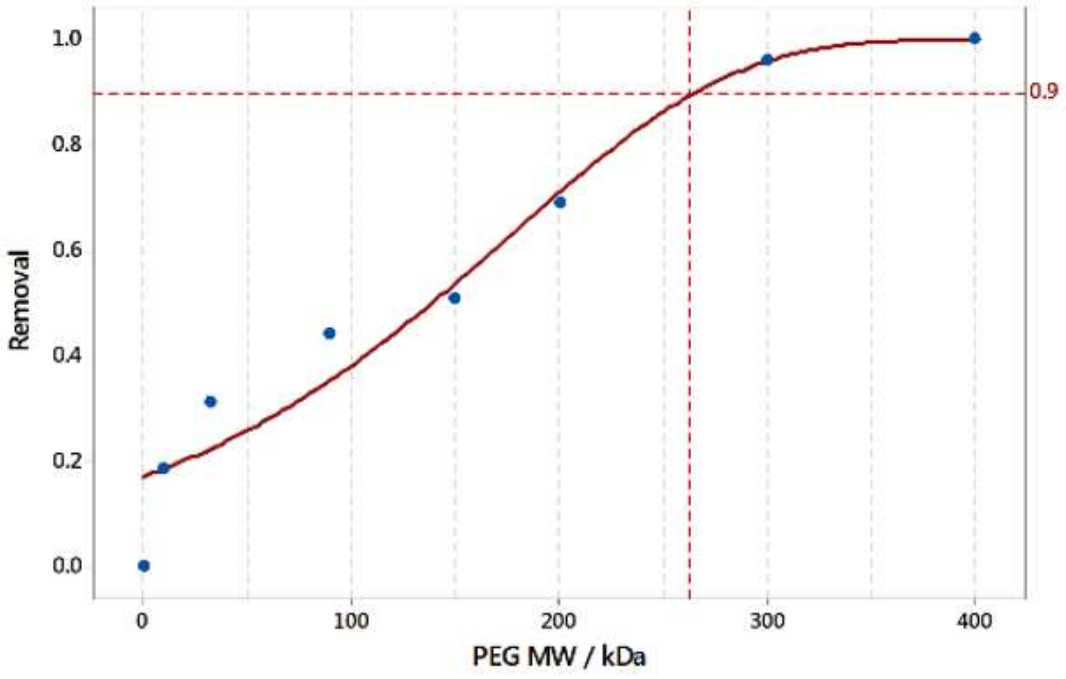

Figure 6. 20% PES membrane MWCO.

Table 6. ANOVA statistical analysis of membranes MWCO.

Source	DF	Adj. SS	Adj. MS	F-Value	P-Value
Factor	1	0.00238	0.002377	0.02	0.894
Error	14	1.81083	0.129345		
Total	15	1.81320			

4. Conclusions

In this work, 16 and 20% PES membranes were compared to verify whether the concentration of polymer can determine a significant difference between them. Based on results obtained, it is possible to observe that the only relevant statistical difference was PWP, which is almost 25% better in the 16% PES membrane, implying a minor energy consumption to produce the same volume of permeate compared to the 20% PES. All other results have no relevant statistical difference among the compared membranes.

Future studies involving membrane modification can benefit from this research and motivate researchers to develop other works with related themes.

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References

[1] Bildyukevich, A. V., Plisko, T. V. Usosky, V. V., The formation of polysulfone hollow fiber membranes by the free fall spinning method, Petroleum Chemistry 56 (2) (2016) 379-400. https://doi.org/10.1134/S0965544116050042.

[2] Kurada, K. V., De, S., Modeling of solution thermodynamics: A method for tuning the properties of blend polymeric membranes, Journal of Membrane Science 540 (2017) 485-495. https://doi.org/10.1016/j.memsci.2017.06.049.

[3] Werber, J. R., Osuji, C. O., Elimelech, M., Materials for next-generation desalination and water purification membranes, Nature Reviews Materials 1 (2016) 16018. https://doi.org/10.1038/natrevmats.2016.18.

[4] Khulbe, K. C., Matsuura, T., Recent progress in polymeric hollow-fibre membrane preparation and applications, Membrane Technology 2016 (7) (2016) 7-13. https://doi.org/10.1016/S0958-2118(16)30149-5.

[5] Simone, S., Galiano, F., Faccini, M., Boerrigter, M. E., Chaumette, C., Drioli, E., Figoli, A., Preparation and

Characterization of Polymeric-Hybrid PES/TiO_2 Hollow Fiber Membranes for Potential Applications in Water Treatment, Fibers 5 (2) (2017) 14. https://doi.org/10.3390/fib5020014.

[6] Lan, P., Wang, W., Structure Morphology of Polyethersulfone Hollow Fiber Membrane via Immersion Precipitation Phase Inversion Process, Applied Mechanics and Materials 152-154 (2012) 574-578. https://doi.org/10.4028/www.scientific.net/AMM.152-154.574.

[7] Ahmad, A. L., Otitoju, T. A., Ooi, B. S., Hollow fiber (HF) membrane fabrication: A review on the effects of solution spinning conditions on morphology and performance, Journal of Industrial and Engineering Chemistry 70 (2019) 35-50. https://doi.org/10.1016/j.jiec.2018.10.005.

[8] Chew, C. M., Aroua, M. K., Hussain, M. A., Advanced process control for ultrafiltration membrane water treatment system, Journal of Cleaner Production 179 (2018) 63-80. https://doi.org/10.1016/j.jclepro.2018.01.075.

[9] Tsehaye, M. T., Velizarov, S., Van der Bruggen, B., Stability of polyethersulfone membranes to oxidative agents: A review, Polymer Degradation and Stability 157 (2018) 15-53. https://doi.org/10.1016/j.polymdegradstab.2018.09.004.

[10] Alsalhy, Q. F., Salih, H. A., Simone, S., Zablouk, M., Drioli, E., Figoli, A., Poly(ether sulfone) (PES) hollow-fiber membranes prepared from various spinning parameters, Desalination 345 (2014) 21-35. https://doi.org/10.1016/j.desal.2014.04.029.

[11] Xu, Z.-L., Qusay, F. A., Polyethersulfone (PES) hollow fiber ultrafiltration membranes prepared by PES/non-solvent/NMP solution, Journal of Membrane Science 233 (1-2) (2004) 101-111. https://doi.org/10.1016/j.memsci.2004.01.005.

[12] Liu, T., Xu, S., Zhang, D., Sourirajan, S., Matsuura, T., Pore size and pore size distribution on the surface of polyethersulfone hollow fiber membranes, Desalination 85 (1) (1991) 1-12. https://doi.org/10.1016/0011-9164(91)85142-H.

[13] Chung, T. S., Hu, X., Effect of air-gap distance on the morphology and thermal properties of polyethersulfone hollow fibers, Journal of Applied Polymer Science 66 (6) (1997) 1067-1077. https://doi.org/10.1002/(SICI)1097-4628(19971107)66:6%3C1067::AID-APP7%3E3.0.CO;2-G.

[14] Thong, Z., Gao, J., Zoe, J. X., Wang, K.-Y., Chung, T.-S., Fabrication of loose outer-selective nanofiltration (NF) polyethersulfone (PES) hollow fibers via single-step spinning process for dye removal, Separation and Purification Technology 192 (2018) 483-490. https://doi.org/10.1016/j.seppur.2017.10.031.

[15] Xu, Z.-L., Qusay, F. A., Effect of Polyethylene Glycol Molecular Weights and Concentrations on Polyethersulfone Hollow Fiber Ultrafiltration Membranes, Journal of Applied Polymer Science 91 (5) (2004) 3398-3407. https://doi.org/10.1002/app.13580.

[16] Chung, T. S., Teoh, S. K., Hu, X., Formation of ultrathin high-performance polyethersulfone hollow-fiber membranes, Journal of Membrane Science 133 (2) (1997) 161-175. https://doi.org/10.1016/S0376-7388(97)00101-4.

[17] Deng, K., Liu, Z., Luo, F., Xie, R., He, X.-H., Jiang, M.-Y., Ju, X.J., Wang, W., Chu, L.-Y., Controllable fabrication of polyethersulfone hollow fiber membranes with a facile double co-axial microfluidic device, Journal of Membrane Science 526 (2017) 9-17. https://doi.org/10.1016/j.memsci.2016.12.012.

[18] Prisciandaro, M., Di Celso, G. M., On the removal of natural organic matter from superficial water by using UF and MF membranes, Desalination and Water Treatment 57 (6) (2016) 2481-2488. https://doi.org/10.1080/19443994.2015.1031184.

[19] Le, N. L., Nunes, S. P., Materials and membrane technologies for water and energy sustainability, Sustainable Materials and Technologies 7 (2016) 1-28. https://doi.org/10.1016/j.susmat.2016.02.001.

[20] Novotna, K., Cermakova, L., Pivokonska, L., Cajthaml, T., Pivokonky, M., Microplastics in drinking water treatment – Current knowledge and research needs, Science of the Total Environment 667 (2019) 730-740. https://doi.org/10.1016/j.scitotenv.2019.02.431.

[21] Wang, Z., Lin, T., Chen, W., Occurrence and removal of microplastics in an advanced drinking water treatment plant (ADWTP), Science of the Total Environment 700 (2020) 134520. https://doi.org/10.1016/j.scitotenv.2019.134520.

[22] Ma, B., Xu, W., Hu, C., Liu, H., Qu, J., Li, L., Characteristics of microplastic removal via coagulation and ultrafiltration during drinking water treatment, Chemical Engineering Journal 359 (2019) 159-167. https://doi.org/10.1016/j.cej.2018.11.155.

[23] Koelmans, A. A., Nor, N. H. M., Hermsen, E., Kooi, M., Mintenig, S. M., De France, J., Microplastics in freshwaters and drinking water: Critical review and assessment of data quality, Water Research 155 (2019) 410-422. https://doi.org/10.1016/j.watres.2019.02.054.

[24] Septianto, F., Lee, M. S. W., Emotional responses to plastic waste: Matching image and message framing in encouraging consumers to reduce plastic consumption, Australasian Marketing Journal 28 (1) (2020) 18-29. https://doi.org/10.1016/j.ausmj.2019.09.002.

[25] Morihama, A. C. D., Mierzwa, J. C., Clay nanoparticles effects on performance and morphology of poly(vinylidene fluoride) membranes, Brazilian Journal of Chemical Engineering 31 (1) (2014) 79-93. https://doi.org/10.1590/S0104-66322014000100009.

[26] Arahman, N., Mulyati, S., Lubis, M. R., Razi, F., Takagi, R., Matsuyama, H., Modification of polyethersulfone hollow fiber membrane with different polymeric additives, Membrane Water Treatment 7 (4) (2016) 355-365. https://doi.org/10.12989/mwt.2016.7.4.355.

[27] Qin, J.-J., Gu, J., Chung, T.-S., Effect of wet and dry-jet wet spinning on the shear-induced orientation during the formation of ultrafiltration hollow fiber membranes, Journal of Membrane Science 182 (1-2) (2001) 57-75. https://doi.org/10.1016/S0376-7388(00)00552-4.

[28] Wan, P., Yin, J., Deng, B., Seven-bore hollow fiber membrane (HFM) for ultrafiltration (UF), Chemical Engineering Research and Design 128 (2017) 240-247. https://doi.org/10.1016/j.cherd.2017.09.019.

[29] Al Aani, S., Wright, C. J., Hilal, N., Investigation of UF membranes fouling and potentials as pre-treatment step in desalination and surface water applications, Desalination 432 (2018) 115-127. https://doi.org/10.1016/j.desal.2018.01.017.

[30] Lalia, B. S., Kochkodan, V., Hashaikeh, R., Hilal, N., A review on membrane fabrication: Structure, properties and performance relationship, Desalination 326 (2013) 77-95. https://doi.org/10.1016/j.desal.2013.06.016.

[31] Van der Bruggen, B., Mänttäri, M., Nyström, M., Drawbacks of applying nanofiltration and how to avoid them: A review, Separation and Purification Technology 63 (2) (2008) 251-263. https://doi.org/10.1016/j.seppur.2008.05.010.

[32] Rodrigues, R., Morihama, A. C. D., Barbosa, I. M., Leocádio, G. N., Mierzwa, J. C., Clay Nanoparticles Composite Membranes Prepared with Three Different Polymers: Performance Evaluation, Journal of Membrane and Separation Technology 7 (2018) 1-11. https://doi.org/10.6000/1929-6037.2018.07.01.

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