A study on lupin beans process wastewater nanofiltration treatment and lupanine recovery

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ABSTRACT

The current study addresses an environmental problem of high impact for food processing industries — the efficient use of water contributing to minimize environmental issues concerning water scarcity and improving industrial process sustainability. Lupin beans are highly nutritious seeds, with an increasing use in vegan products. Their characteristic bitter flavor is conferred by the presence of toxic alkaloids, namely lupanine. Although being a rainfed plant with low water requirements, the industrial process to make these beans edible, removing such alkaloids, uses high amounts of fresh water. Nanofiltration, an easy scalable operation, is here investigated for purification of this wastewater, while retaining different organic species, including lupanine, on the retentate. The membrane selected, NF270, present a trans-membrane flux of 33 L h^{-1}·m^{-2} and membrane rejections for lupanine and total organic species of 99.5% and 94.1%, respectively. Further purification of lupanine by solvent extraction and/or resin adsorption was investigated. Amberlite XAD-16 resin and ethyl acetate were selected as promising adsorber and extractant solvent, respectively, for lupanine purification. Overall, the process suggested is able to reclaim around 80% of the wastewater as a water stream with a purity high enough to be recycled in-situ, while 95.4% of lupanine with 78% purity can be isolated corresponding to around 9 Kg of lupanine natural product per ton of dry beans processed. Lupanine conversion to sparteine by reduction using NaBH$_4$/I$_2$ and subsequent distillation under reduced pressure allows the isolation of sparteine in 60% yield and in >95% purity.

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1. Introduction

Lupin beans are legume seeds rich in proteins, currently explored in industry and as part of animal and human diets being mainly used as snack in the Mediterranean region (Huyghe, 1997; Lucas et al., 2015; Mitchell and Shammet, 2008; Gavrilin et al., 2006; Ruiz-López et al., 2019; Prusinski, 2017). Lupin beans characteristic bitterness, conferred by the presence of toxic alkaloids, implies that they have to be properly processed before becoming safely edible (Ganzera et al., 2010). Although being a rainfed plant,

with low water requirements (Sulas et al., 2016), the debittering process of the lupin beans uses large volumes of fresh water and briefly consists in four steps: dry beans hydration, swollen beans cooking, boiled beans sweetening (i.e. a debittering phase, by adding fresh water until the bitter taste and the corresponding alkaloids are removed from the beans, Scheme 1) and a salting final stage for preservation (Carmali et al., 2010). While this process is usually performed in batch, the debittering step, where the largest amount of water of the entire process is consumed, can be performed continuously or in sequential washing steps. Although, from the point of view of agriculture lupin beans crops are not water demanding species (van de Noort, 2017), as mentioned, its industrial processing requires the use of high volumes of fresh water that result in polluted waste streams. Water scarcity caused by water pollution, for example, poses a worldwide challenge to humanity that struggles for fresh water supplies for populations (Pimentel et al., 2004; Inyinbor et al., 2018). Since lupin bean
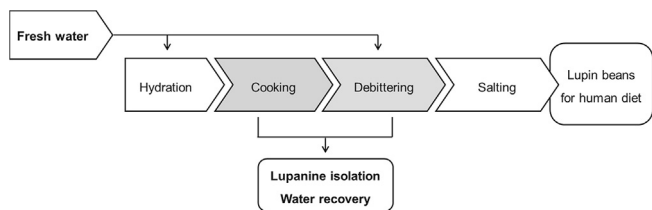
Scheme 1. General representation of industrial lupin beans processing.

processing is water intensive, recycling the fresh water used in the debittering step is therefore of paramount importance to contribute with savings on this limited natural resource and for the process sustainability.

The wastewater from lupin beans industrial process contains oligosaccharides, amino acids, lipids, proteins and alkaloids that are extracted from the beans (Santana et al., 2002). The main alkaloid species present is lupanine (Fig. 1) (Wink et al., 1982, 1987; Hamed and Ayoub, 2015). This compound has been evaluated in rats for the treatment of type 2 diabetes (Zambrana et al., 2018; Lopez et al., 2004; Bobkiewicz-Koslowska et al., 2007), for its effects in the central nervous system (Pothier et al., 1998), and is the starting material for the synthesis of other alkaloids (Wlodarczak et al., 2010; Maulide et al., 2014), namely sparteine (Fig. 1), which is a known building block for the pharmaceutical industry being used as a chiral selector in synthetic procedures (Jasiewicz et al., 2011; Chuzel and Riant, 2005).

Lupanine and sparteine have a very complex chemical structure, making their synthesis or chemical modification quite challenging. Therefore, isolating lupanine from an industrial waste, where it is readily available in significant amounts, is an opportunity worth to be investigated. The efficient treatment of wastewater, for further reuse in the debittering step, and isolation of lupanine from other species present in the organic rich waste stream, is challenging. Several lupin beans debittering processes have been investigated and attempted in bench scale using microorganisms or extraction with organic solvents (e.g. hexane, ethanol, dichloromethane) or alkali compounds (e.g. NaHCO₃), at different temperatures and soaking times (Carvajal-Larenas et al., 2013, 2016; Erbas, 2010; Ertas and Bilgiçli, 2014; Hamed and Ayoub, 2015). However, presently, the only industrial food-grade method for lupin beans debittering relies on the use of large amounts of water generating about 10−20 L of lupanine rich wastewater per Kg of dry lupin beans. Nevertheless, studies dealing with lupin beans industrial wastewaters are scarce in the literature. As an example, Carmali et al. describe a possible strategy for lupanine recovery from industrial lupin beans wastewaters using osmotic evaporation followed by solvent extraction with ethyl ether from a basic solid medium, with only 18.5% of yield (Carmali et al., 2010).

The current work aims to define which are the most suitable unit operations to be used on a process able to simultaneously: i) treat the industrial wastewater and minimize freshwater consumption, and ii) obtain a crude rich in lupanine for further

valorization by chemical derivatization, for example. To achieve this, the use of nanofiltration (NF), solvent extraction and resin adsorption were experimentally assessed at bench scale using industrial wastewaters from lupin beans processing. In NF experiments, several membranes were assessed in order to obtain clean water as permeate and a lupanine rich retentate. Several organic solvents and commercially available resins were evaluated in order to recover and isolate lupanine from the NF retentate lupanine rich fraction, considering minimal contamination with other organic species present in the wastewater. To minimize potential environmental impact, additionally to separation efficiency, the toxicities of the organic solvents and from the additional wastes generated were taken into account on decisions concerning solvent selection (Alder et al., 2016).

2. Materials and methods

2.1. Materials

Heptane, hexane, toluene, diethyl ether (Et₂O), absolute ethanol (EtOH), tetrahydrofuran (THF), hydrochloric acid (HCl) 37% aqueous solution, ethyl acetate (EtOAc) and, dichloromethane (DCM), methanol (MeOH) and acetonitrile (MeCN) HPLC grade were purchased from Fisher Scientific. Methyl tert-butyl ether (MTBE) was purchased from Lab-Scan. Methyl isobutyl ketone (MIBK) was purchased from Acros Organics. 1-Octanol, sodium hydroxide (NaOH) pellets, sodium sulfate (Na₂SO₄) and potassium chloride (KCl) were purchased from Merck. Potassium hydroxide (KOH) pellets and sodium phosphate dibasic (Na₂HPO₄) were purchased from Panreac. Amberlite resins (IRC 50, IRC 7481, IRA 68 and IRA 458-Cl) were purchased from Rohm and Haas. 1-Butanol, Amberlite resins (IRC 86, IRA 400-Cl, IRA 410, XAD-1, XAD-7 and XAD-16), Amberlyst resins (16 and 36), Dowex MAC-3 resin and CDCl₃ (99.8%) used to record ¹H and ¹³C NMR spectra were purchased form Sigma-Aldrich. The resins AG-50W-X2 and AG-50W-X8 were purchased from Bio-Rad. The resin Amberlite CG-400 was purchased from BDH Chemicals. The resin Dowex 1X8-50 was purchased from Alfa Aesar. The resin Purolite PD206 from Purolite was kindly provided by Neoquímica S.A., Portugal.

2.2. Analytical methods

Chemical oxygen demand (COD) was measured as described elsewhere (Himebaugh and Smith, 1979). Conductivity and pH were measured with a portable WD-35607-20 conductivity meter (Oakton, USA) and a 69 pH meter (Metrohm), respectively. ¹H and ¹³C NMR spectra were obtained on a Bruker spectrometer MX300 operating at 300 MHz.

Lupanine was quantified using a Hitachi LaChrom HPLC system at room temperature with UV detection at 220 nm and a reversed-phase Kinetex EVO C18 100 Å column (5 μm, 250 mm × 4.6 mm, Phenomenex). lupanine samples were basified with KOH (pH 13−13.5), centrifuged using a 1-15P microcentrifuge (Sigma) at 6000 rpm for 4 min and filtered with nylon syringe filters (13 mm diameter and 0.22 μm pore size, Tecnocroma). The method was isocratic for 25 min, at 1 mL/min with the mobile phase as 15% MeCN and 85% Na₂HPO₄ (1.8 g/L) buffer adjusted with NaOH at pH 10.5, with 20 μL injection volume.

2.3. Wastewater

Industrial lupin beans wastewater was kindly provided by Tremoceira M. Ferreira Bastos Lda. (TMFB), Portugal. Two types of effluent samples were considered in this study:
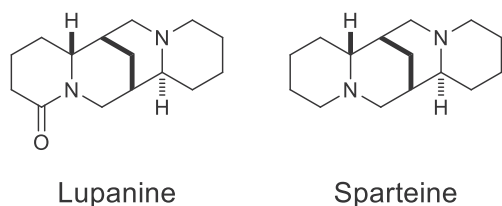

Fig. 1. Structures of lupanine and sparteine alkaloids.

i) a cooking step, corresponding to the wastewater obtained after hydration, cooking and cooling stages of the beans. This wastewater fraction is obtained after cooking the beans, and will be referred to as lupin bean cooking wastewater phase;

ii) and a debittering step wastewater, corresponding to an industrial stream obtained from the elution of lupanine from the beans, with addition of fresh water. It is important to mention that, lupanine and organic matter contents decrease along this operation. Therefore, in the current study we used a composite solution representative of the whole debittering step.

Characterization of both effluent samples can be found in Table 1. COD is used in this study as a metric to assess the amount of organic matter in the wastewater, which include lupanine itself.

2.4. Nanofiltration experiments

The following NF membranes from Dow Filmtec, purchased from Lenntech, were tested: NF270, NF200 and NF90. Membrane characteristics can be found in Table 2 (Table S1). The experiments were performed on concentration or total recirculation mode (for further details on operation modes see Supporting Information, Scheme S1).

Flux (J) and Permeability (Lp) were calculated according to equations (1) and (2), considering a total membrane area of $0.0026 \ m^2$.

$$J \ (L/h^1 \cdot m^2) = {}_{Flow} \ rate \ (L/h^1) \ / \ Membrane \ area \ (m^2) \tag{1}$$

$$Lp \ (L/h^1 \cdot m^2 \cdot bar^1) = J \ (L/h^1 \cdot m^2) \ / \ P \ (bar) \tag{2}$$

COD and lupanine rejections were calculated using equations (3) and (4),

$$Rejection_{COD} \ (\%) = (COD_{feed} - COD_{permeate}) \ / \ COD_{feed} \ x \ 100 \tag{3}$$

$$Rejection_{lupanine} \ (\%) = (lupanine_{feed} - lupanine_{permeate}) / \ lupanine_{feed} \ x \ 100 \tag{4}$$

where COD_{feed} is the COD value in the composite wastewater sample and $COD_{permeate}$ is the COD value determined for the permeate recovered in the end of the NF experiment. $Lupanine_{feed}$ is the concentration of lupanine in the reservoir tank, and $lupanine_{permeate}$ is the concentration of lupanine in the permeate.

2.5. Resin adsorption

2.5.1. Batch experiments

Eighteen different resins were assessed with samples of pure lupanine in water, and with the cooking wastewater sample as received (at pH 4) or after pH adjusted with NaOH pellets to pH 11 and centrifugation (5 min at 6000 rpm). The characteristics of the adsorbers can be found in Table S2. The percentage of lupanine binding (%) and the adsorption capacity (Q) for each resin was calculated from equations (5) and (6), respectively:

$$Lupanine \ binding \ (\%) = (C_{L,i} - C_{L,f}) \ / \ C_{L,i} \ x \ 100 \tag{5}$$

Table 1
Wastewater samples from lupin bean industrial processing.

	Lupanine (g/L)	COD (gO_2/L)
Cooking stage	3.26 ± 0.07	27.85 ± 0.90
Composite sample	0.40 ± 0.02	5.31 ± 0.55

Table 2
Characterization of the nanofiltration (NF) membranes assessed in this study: NF90, NF200 and NF270.

Membrane	MWCO	Salt rejection
NF90	257–330 Da	>98% MgSO$_4$ 90–96% NaCl
NF200	~200 Da	>98% MgSO$_4$ 50% NaCl
NF270	200–400 Da	>98% MgSO$_4$ 50% NaCl

$$Q \ (mg/g) = (C_{L,i} - C_{L,f}) \ / \ M \ x \ V \tag{6}$$

where, $C_{L,i}$ corresponds to the concentration of lupanine in the sample, $C_{L,f}$ is the final lupanine concentration, V is the volume of sample used (1.5 mL) and M is the mass of resin used (150 mg).

2.5.2. Lupanine recovery experiments

For XAD-16 resin, 900 μL of absolute ethanol were added to the Eppendorf tubes containing the resins after adsorption. The mixtures were left at 200 rpm at room temperature for 15 h. After this time, the mixtures were centrifuged at 6000 rpm for 4 min for resin separation. The solvent was evaporated at room temperature and the volume refilled with water before lupanine HPLC analysis protocol. For MAC 3 resin, 750 μL of a NaOH 10% (w/w) solution were added to the Eppendorf tubes containing the resins after adsorption. The mixtures were left at 200 rpm at room temperature for 15 h. After this time, the mixtures were centrifuged at 6000 rpm for 4 min for resin separation and the supernatant was analyzed for lupanine content as described above.

2.5.3. Resin regeneration experiments

For XAD-16 and MAC-3, after lupanine recovery assays, 1 mL of distilled water and 750 μL of HCl 10% (w/w) were added, respectively, to the resins (150 mg) and left stirring at 200 rpm at room temperature for 15 h. After this time, the mixtures were centrifuged at 6000 rpm for 4 min, the resins removed, and the supernatant was collected and submitted to lupanine analysis protocol (no lupanine was detected). The resins were then used in several cycles, each including as described above, one step of adsorption, one step of lupanine recovery and one step of regeneration to assess potential re-use of resins.

2.5.4. Column chromatography

A glass column chromatography, with a diameter of around 1 cm plugged with cotton, was packed with 15 g of Amberlite XAD-16 resin and preconditioned with distilled water, to give a height of c.a. 9 cm 100 mL of cooking wastewater sample was centrifuged for 30 min at 6000 rpm and passed through the column for lupanine adsorption with a constant flow around 2 mL/min, followed by 60 mL of EtOH for lupanine recovery and 100 mL of distilled water for resin regeneration at a flow around 1 mL/min 2 mL aliquots were collected for lupanine and COD measurement. A new binding/recovery/regeneration cycle was performed by passing the same volumes of wastewater, EtOH and distilled water through the column, respectively. The EtOH fractions (15 mL) with high lupanine content were processed as described in section 2.5.2. for lupanine further purification by solvent extraction and for lupanine and COD measurements.

2.6. Liquid-liquid extraction experiments

Ten organic solvents from different chemical classes were assessed: DCM, MTBE, MIBK, heptane, hexane, toluene, Et$_2$O, 1-

octanol, 1-butanol and EtOAc. 4 mL of cooking stage wastewater (previously basified to pH 12−13 with NaOH) and of aqueous solutions of pure lupanine (c.a. 3.26 g/L) were extracted with two successive extractions, using 2 mL of each solvent. In each extraction, a vertical vortex (IKA) was used for 2−3 min with settling times of 8−10 min. Lupanine extraction efficiency was calculated by equation 7:

$$\text{Extraction efficiency (\%)} = [(C_{L,aq0} \times V_{aq0}) - (C_{L,aq} \times V_{aq})] / (V_{aq0} \times C_{L,aq0}) \times 100 \qquad (7)$$

where $C_{L,aq0}$ and V_{aq0} are the initial concentration of lupanine in lupin beans cooking wastewater and the initial volume of the aqueous phase and $C_{L,aq}$ and V_{aq} are the concentration of lupanine and the volume of the aqueous phase after the two extractions, respectively. For details concerning blank experiments for solvent extraction, please see Supporting Information file.

Purity of lupanine for solvent extraction experiments was determined by equation 8:

$$\text{Purity (\%)} = COD_{lupanine} / COD_{org.phase} \times 100 \qquad (8)$$

where $COD_{lupanine}$ is calculated as 2.33 x V_{aq0} x ($C_{L,aq0} - C_{L,aq}$) and $COD_{org.phase}$ is calculated as V_{aq0} x [$COD_{aq0} - (COD_{aq} - COD_{aq0})$] where COD_{aq0} is the COD of the wastewater sample and COD_{aq} is the COD of the aqueous phase of the extraction. The COD of an aqueous sample of pure lupanine (at 1 g/L) was quantified resulting on a value of 2.33 ± 0.27 gO_2/L.

Liqui-Liquid extractions for MTBE and EtOAc were also performed at a larger scale with 150 mL of cooking wastewater sample in two successive extractions with 75 mL of organic solvent.

Lupanine extraction with MTBE and EtOAc was optimized using 4 mL of cooking wastewater, basified to pH 12−13 with NaOH, extracted 4 times with 4 mL of MTBE or 3 times with 4 mL of EtOAc, as described in section 2.6. The aqueous phases were used for COD and lupanine quantification as described in section 2.2.

2.7. Lupanine extraction from column chromatography fractions

The column fractions, obtained in EtOH (see section 2.5.4), in a total of 15 mL, were collected and evaporated to dryness at room temperature. The residue was dissolved in 15 mL of distilled water, the pH was adjusted to 12−13 with NaOH pellets and the resulting solution was successively extracted twice with 7.5 mL of MTBE or EtOAc. The aqueous phases were processed as described in section 2.2. for lupanine quantification and COD measurements.

2.8. Lupanine conversion in sparteine

To a stirred solution of lupanine (5.0 g, 20 mmol) in THF (200 mL) was added $NaBH_4$ (0.76 g, 1.0 eq) and I_2 (2.55 g, 0.5 eq). The mixture was stirred under reflux for 16 h and then cooled to room temperature. MeOH (25 mL) was added and the mixture was subsequently poured into 50 mL of aqueous NaOH (1 M). The resulting solution was extracted with Et_2O (3 × 200 mL), the organic extracts were dried over Na_2SO_4, concentrated under vacuum and then distilled in a Kugelrohr (190-220 °C/0.7−1 mbar) from Buchi to afford sparteine (2.8 g, with 60% reaction yield) as a slightly yellow liquid, pure by ^1H and ^{13}C NMR analysis.

3. Results and discussion

3.1. Nanofiltration experiments

The problem to be solved with NF is to obtain clean water in the

permeate and a retentate rich in lupanine. The nanofiltration also works as an operation to concentrate lupanine in a reduced wastewater volume, allowing smaller further processing unit operations, such as solvent extraction or resin adsorption, for lupanine isolation.

Previously to the NF experiments, a centrifugation was performed, with no influence on COD content of the wastewater, to eliminate suspended particles, which on process implementation may be replaced by an in-line pre-filtration.

The membranes studied in this work, NF90, NF200 and NF270 (Table 2), were evaluated on their flux, flow, permeability and, COD and lupanine rejections. The detailed experimental data obtained can be found in Table S3. Initial experiments were performed using around 400 mL of cooking wastewater, concentrated until 50−60% of the initial volume at two different pH values, 4 and 11. The characteristic pH of the wastewater is 4, however, lupanine has a pKa of 9.1 (Fig. 2) (Mende and Wink, 1987). Therefore, it was decided to evaluate if lupanine rejection was dependent on its protonation state. pH and conductivity were also determined for the permeate and retentate obtained in the end of the experiments.

From Fig. 3 it is possible to observe that, from the membranes studied, NF270 showed the highest rejection for lupanine and organic matter (COD) with values higher than 95% together with the highest permeability around 3.5 L/m^2·h·bar at pH 4, making this the selected membrane for lupin beans cooking wastewater NF processes. However, concerning conductivity, this membrane presented a rejection of 52% (Fig. S1) at pH 4, while NF90 membrane presented a rejection of 92% at the same pH. In this case, the water obtained in the permeate with the NF270 membrane may need further polishing to reduce ion content before being reused, for example, in situ for reduction of freshwater demand in the debittering stage.

NF270 membrane was first assessed in concentration mode using the composite wastewater sample for estimation of flux over time up to a concentration percentage (here defined as permeate/feed ratio). This sample is representative of the debittering step where the larger volume of wastewater is generated. The flux across the membrane represented on Fig. 4 (top left panel) is illustrative of several assays performed and shows that in spite of flux decline observed, probably due to an increase on osmotic pressure and viscosity, the observed values are maintained above about 30 L h^{-1}·m^{-2} up to concentration percentages as high as 80%. In these assays, using the debittering composite wastewater sample, a lupanine concentration and a COD of about 2 g/L and 27 gO_2/L respectively, were achieved, which are in the same concentration range of the cooking wastewater sample with values of 3.26 g/L and 27.85 gO_2/L for lupanine and COD, respectively (Table 1 and Fig. 4, top right).

A full recirculation experiment was performed for further investigation of the robustness of this operation and as preliminary assessment of further flux decline due to potential adsorption events such as fouling formation. The full recirculation experiments were performed using the more concentrated sample of the cooking step over 16 h (after an initial concentration percentage of 25%).

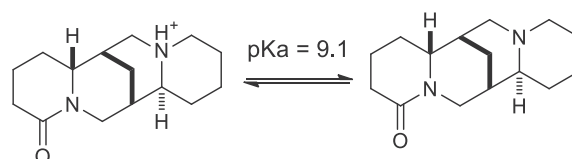From the results of the full recirculation mode experiment

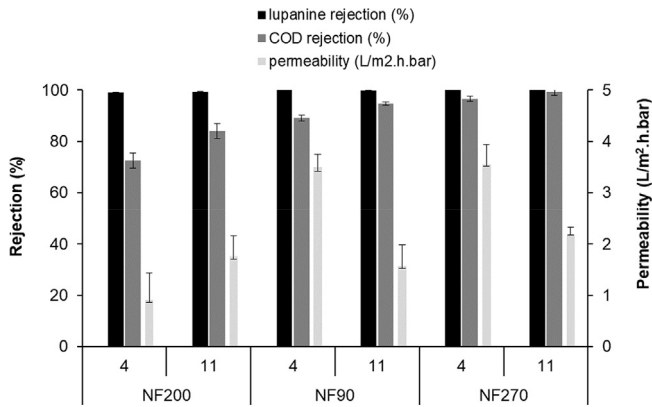**Fig. 2.** pKa and protonation states of lupanine.

Fig. 3. Lupanine and COD rejections in the primary axis; permeability in the secondary axis for nanofiltration membranes. Rejections measured for a concentration factor of 66% at a pressure of 24 bar.

(Fig. 4, bottom) it is possible to observe that, even on more stringent conditions, the membrane flux decline over time was not significant, remaining above 25 L h^{-1}.m^{-2}. This result is a preliminary indication of the robustness of the membrane nanofiltration operation, with the species present not posing fouling issues over time. Note that, the characteristics of the initial and retentate solutions over the assays of the full recirculation of the cooking water are similar to the ones of the final retentate solutions obtained on the concentration mode of the composite sample. Following the same rational and for practical reasons, the lupin beans cooking wastewater was directly used on screening experiments for solvent extraction and resin adsorption steps in which the retentate of the nanofiltration of the total wastewater, obtained from the debittering stage, would be used.

Those following up operations (resin adsorption and solvent liquid-liquid extraction) could benefit from further volume reduction and concentration in lupanine beyond the range of 2–5 g/L. To assess such option a sample of the cooking phase was also subjected to an assay on the concentration mode. However, such option poses a more challenging case with a steep flux decline (Fig. S2), probably due to the high content in organic matter, increase on osmotic pressure and viscosity, as well as possible fouling agents. Still, the membrane maintained a high selectivity, retaining effectively lupanine to concentrations as high as 9.5 g/L, with virtually no lupanine found in the permeate (Fig. S2). Again, NF270 membrane also showed a good performance in retaining organic matter reaching high COD levels in the retentate comparing with the feed (Fig. S2).

Importantly, the lupanine on the permeate was virtually zero on the assays on the recirculation mode with cooking water and 1.6 and 6.2 mg/L on the assays on concentration mode using cooking water and composite sample of the debittering wastewater. As mentioned, lupanine concentration decreases over the debittering step from high to residual values, where lupin beans are almost ready for consumption. Lupanine concentrations obtained on the permeate, of the several nanofiltrations performed, are lower than lupanine EC50 value and lower than the residual lupanine content obtained in the end of lupin beans debittering step. Therefore, from a logic of water re-use within the same process, the water purified on the permeate fraction can be potentially recycled for lupin beans debittering. The COD measured in the permeate was higher than the legislated reference value of 5 mgO$_2$/L for water quality for human consumption to be used in a company of the food sector (Council Directive, 1998; Decree-Law, 306/2007). This means that eventual further polishing of the permeate may need to be performed before its use in-situ for water recycling. However, since the organic species present in the wastewater are inherent to the process, from which it is originated, upon proper qualification of edibility of the lupin beans generated, these levels of COD are low enough to not impair the reutilization of this treated water within the same industrial process. In this case, the water reclaiming would be a water saving measure and at the same time it would contribute to reduce the pollutant charge sent to the municipal wastewater treatment plant.
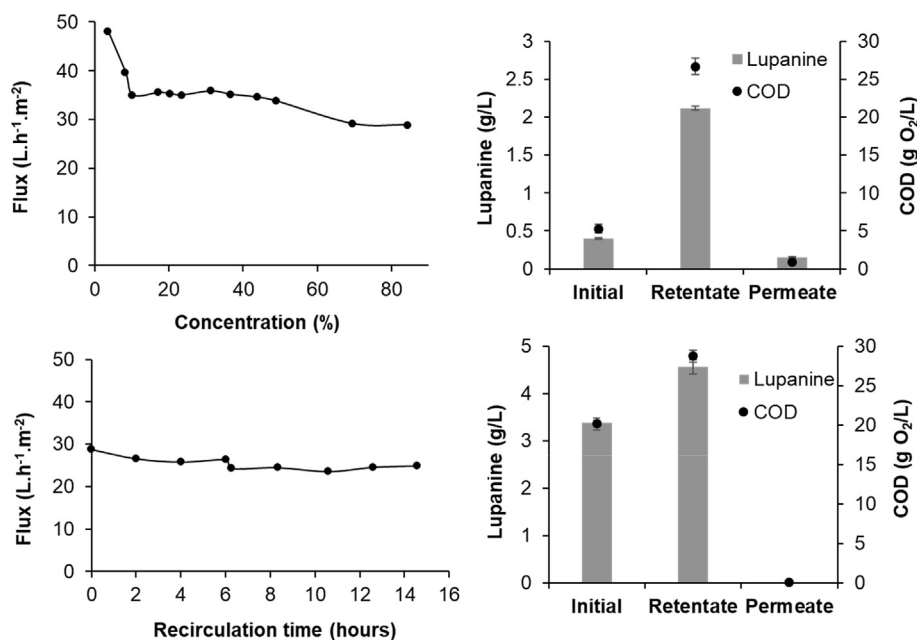

Fig. 4. Nanofiltration experiments with NF270 membrane at 20 bar: Top - concentration experiment with the composite sample; Bottom - recirculation experiment with the cooking phase. Right: Lupanine and COD contents of initial samples, retentate and permeate of NF experiments for composite sample (top) and cooking phase (bottom).

3.2. Resin adsorption experiments

The technical issue addressed in this section is the isolation of lupanine from the retentate of NF using commercially available resins in an adsorption step. At the same time, it is expected the recovery of lupanine with minimal organic matter contamination. Lupanine binding and recovery for different adsorbers, COD elimination, and resin regeneration and recyclability were assessed. Binding assays were performed for 18 commercially available resins with different chemical functionalities using lupin beans cooking wastewater, without any pre-treatment, and the results were compared with pure lupanine samples prepared in water, at the same concentration range. From Fig. 5 (top) it is possible to observe that the resins with higher lupanine adsorption corresponded to strong and weak acid cation exchangers (AG 50W-X2, AG 50W-X8, Amberlyst 36, Amberlyst 16, Purolite PD206, Amberlite IRC 50, Amberlite IRC 86 and Dowex MAC-3) and also to the polymeric resin Amberlite XAD-16.

Lupanine has a pKa value around 9.1 (Mende and Wink, 1987), and the wastewater pH is around 4, which means that lupanine is
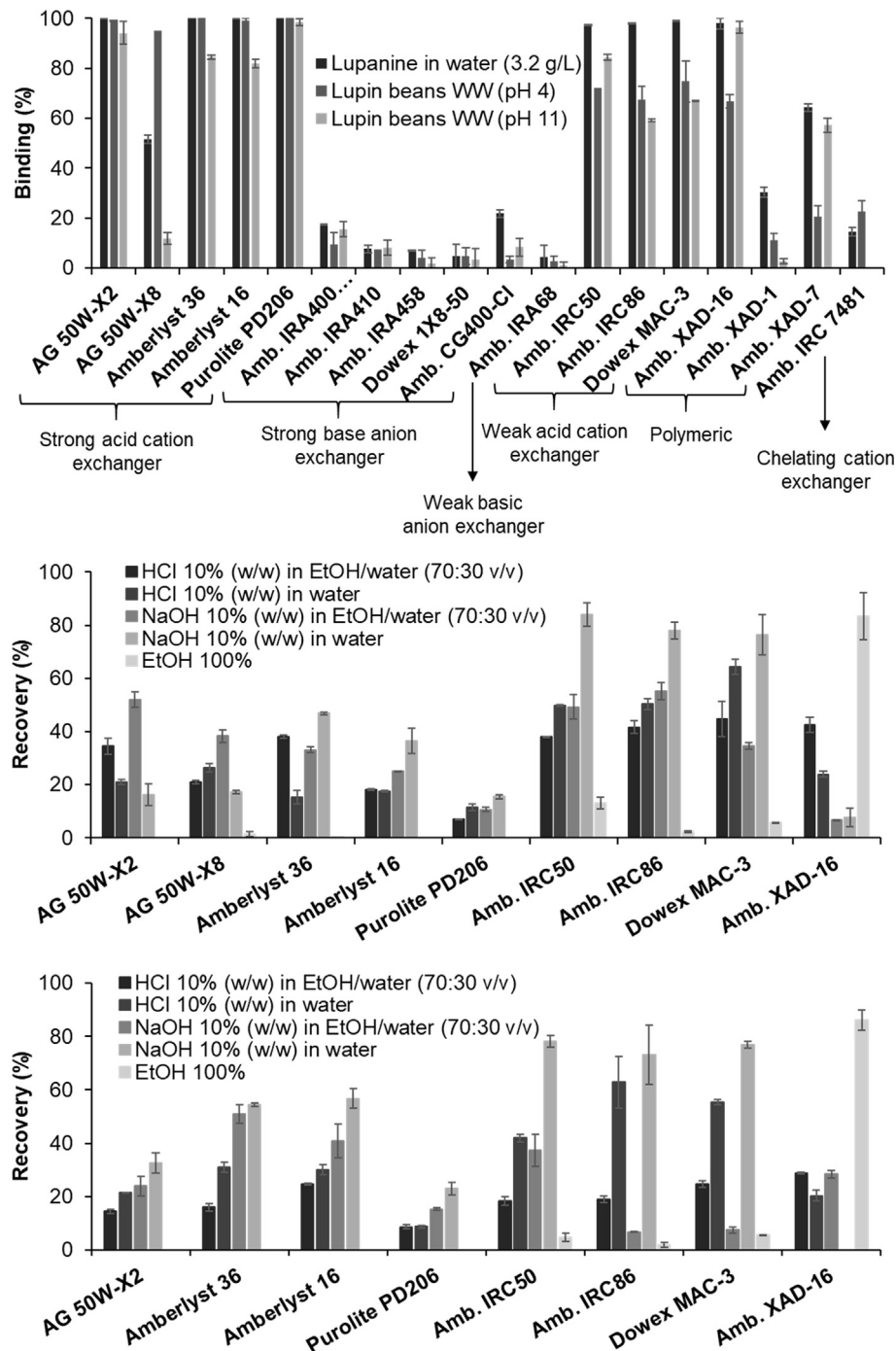

Fig. 5. Top: Lupanine binding for several commercially available resins for pure samples in water and lupin beans cooking wastewater at pH 4 and pH 11. Middle: Lupanine recovery from acidic resins and the polymeric resin Amberlite XAD-16 after elution at pH 4. Bottom: Lupanine recovery from acidic resins and the polymeric resin Amberlite XAD-16 after elution at pH 11. Amb. = Amberlite.

present as lupaninium ion in those solutions (Fig. 2). Thus, lupanine will have a positive charge that will exchange with the protons of the sulfonic or carboxylic acid groups of these cationic exchanger resins. In order to test the adsorbers performance towards lupanine in its neutral form, the same adsorption assays were also performed with the pH of the wastewater adjusted to 11 (Fig. 5).

As expected, the anionic exchange resins were not efficient for lupanine binding, because the resin functional groups are positively charged (Table S2) and there is a repulsion between these groups and the positively charged lupaninium molecules. Moreover, no interaction with neutral lupanine molecules at higher pH was expected with the functional groups of such resins. However, a low binding could still be obtained in some cases, which may be a result of non-ionic interactions between lupanine and the polymeric matrix of the resins.

Acrylic polymers are able to form hydrogen bonds and styrene cross-linked with divinylbenzene copolymers can interact through hydrophobic effects. The polymeric resin that promoted higher adsorption of lupanine was Amberlite XAD-16 with a hydrophobic polymeric chain. It interacts with organic compounds (like lupanine) through hydrophobic and polar effects, due to the presence of pi-pi, namely aromatic, bonds. Amberlite XAD-7 is composed of an acrylic polymer which means that there is the possibility of hydrogen bonding between lupanine and the resin. Since hydrogen bonds are stronger interactions than hydrophobic or polar interactions, a higher binding would be expected for XAD-7. However, while the pore diameter is similar for both resins (90 Å for XAD-7 and 100 Å for XAD-16), the surface area of XAD-16 (900 m^2/g) is about the double of the one of XAD-7 (450 m^2/g) (Sigma, 2019) which can result on higher binding capacity and explain the higher performance of XAD-16 in comparison with XAD-7.

3.2.1. Lupanine recovery from resins

Recovery of lupanine from the adsorbers was preliminarily assessed for the resins presenting higher lupanine binding, namely, strong and weak acid cation exchangers and the polymeric adsorber Amberlite XAD-16. Five different washing solutions were tested: HCl 10% (w/w) in water, NaOH 10% (w/w) in water, HCl 10% (w/w) in EtOH/water (70:30 v/v), NaOH 10% (w/w) in EtOH/water (70:30 v/v) and absolute EtOH. While the use of resins represents an attempt to develop a solvent free process for lupanine isolation, the specific use of EtOH is interesting from process intensity perspective, as it is one of the solvents that can be used for further lupanine resolution by diastereomeric recrystallization (Maulide et al., 2014). The use of NaOH and HCl solutions aims to explore ionic interactions, while the use of EtOH aims to explore the use of weaker interactions (hydrophobic or dipole-dipole). While the use of EtOH for recovery of lupanine from the ionic exchange resins proved not to be efficient (Fig. 5), the use of this solvent was very useful to recover lupanine adsorbed on XAD-16 resin. In general, treatment of the acidic resins with NaOH in water seemed to be

more efficient than with HCl. Sodium (Na^+) ions compete with protonated lupanine to bind the resin, lupanine (in neutral form) dissociates from the resin and moves to solution. In the case of HCl, it dissociates in water, and the protons (H^+) will protonate the sulfonic or carboxylic groups from the resins, allowing protonated lupanine to be recovered. Aqueous solutions of HCl and NaOH with EtOH were unsuccessful on promoting significant increases on lupanine recovery from those resins.

Considering the results of lupanine binding and recovery, the results point out for a strategy employing as adsorber the weak acid cation exchanger resins Amberlite IRC50, Amberlite IRC86 and Dowex MAC-3, or the polymeric adsorber resin Amberlite XAD-16. Although the strong acid cation exchanger resins presented a lupanine binding closer to 100%, its recovery was more efficient for the weak acid cation exchanger resins (around 80% of lupanine recovered for 70% of binding). Still, it should be noted that the basification of wastewater brings significant improvements for binding of lupanine on its neutral form to Amberlite XAD-16 up to values of 96% (recovery of 89.5%). Moreover, the resins Amberlite IRC50 and IRC86 were discontinued, being substituted by the resin Dowex MAC-3, presenting a similar performance. Therefore, the following results will concern only MAC-3 and XAD-16 resins.

The ability of the resins to separate lupanine from the total organic matter, was also assessed. Ideally, during resin batch adsorption assays, while the lupanine will be retained in the resin, the other organic matter would remain dissolved in solution. The COD remaining in solution after adsorption assays using MAC-3 and XAD 16 resins was measured and results for estimated total adsorbed organic species (expressed as COD) are summarized in Table 3. It was observed that, around (19−32)% of COD remained adsorbed to the resin from which some contribution is due to lupanine itself. The purity of the combined steps of lupanine adsorption and recovery was estimated as the ratio of COD on the recovery solution due to lupanine by the total COD measured for that solution (after full evaporation of EtOH used as recovery solvent). After the recovery step, although the purity of lupanine is similar for both adsorbers at both pH values, (52−65)%, derived by the higher binding of lupanine on its neutral form to XAD-16 resin at pH 11, this adsorber presents a higher performance with an overall yield around 86% compared with only around 57% for MAC-3 resin at pH 4, for lupanine recovery from the wastewater.

3.2.2. Resin regeneration and recyclability

To assess the recyclability of the two selected resins, 3 binding/regeneration cycles were performed for XAD-16 and MAC-3 resins. Results (Fig. S3) show that both adsorbers present a stable behavior throughout the three binding/recovery cycles performed, with lupanine binding remaining constant. For MAC-3 resin, lupanine recovery is performed with an aqueous solution of NaOH in which the Na^+ ions exchange with lupanine and stay in the polymeric matrix. After this step, resin regeneration is performed with HCl in

Table 3
Lupanine binding, recovery, overall yield and purity and COD adsorbed for MAC-3 and XAD-16 resins at pH 4 and pH 11 for lupin beans cooking wastewater.

			Lupanine				
		pH	Binding (%)	Recovered (%)	Overall yield (%)	Purity (%)[a]	$COD_{in\ resin}$ after binding (%)**
Batch Binding[b]	**MAC-3**	4	74.74 ± 8.22	76.31 ± 7.63	57.03 ± 8.48	52.42 ± 4.14	28.48 ± 3.04
		11	66.88 ± 0.28	76.83 ± 1.22	51.38 ± 0.84	65.71 ± 1.61	32.69 ± 3.65
	XAD-16	4	66.77 ± 2.70	83.38 ± 8.83	55.67 ± 6.31	62.98 ± 0.62	19.35 ± 3.04
		11	96.29 ± 2.40	89.47 ± 4.22	86.15 ± 4.60	63.11 ± 0.65	24.52 ± 4.26
CC[c]	**XAD-16**	11	97.80 ± 1.13	79.43 ± 2.74	77.68 ± 3.09	48.00 ± 2.42	54.07 ± 2.09

[a] $COD_{lupanine}/COD_{total}$ after lupanine recovery (%). ** includes COD due to lupanine itself.
[b] Assays at 100 $mg_{resin}/mL_{wastewater}$.
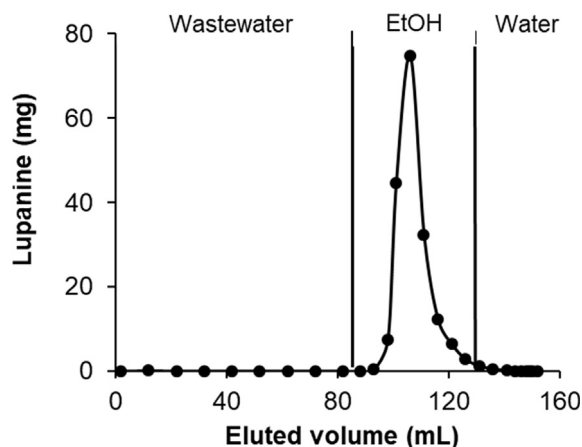[c] column chromatography at 150 $mg_{resin}/mL_{wastewater}$.

Fig. 6. Column chromatographic profile using XAD-16 resin for the lupin beans cooking wastewater at pH 11 through binding/recovery/regeneration.

water to swap the Na^+ ions back to H^+, promoting the protonation and consequent regeneration of the resin. Regarding XAD-16 resin, lupanine recovery is performed using EtOH, that has high affinity to the polymeric matrix. By the end of the recovery assay, the resin is saturated in the solvent molecules, and regeneration is performed by washing with water, to remove the EtOH molecules. Further details on isotherm and kinetic studies for the wastewater with XAD-16 and MAC-3 resins at room temperature can be found in Supporting information.

3.3. Column chromatography

Column chromatography was performed for the cooking wastewater using XAD-16 resin to check the results obtained in binding batch experiments, so that a small volume had to be processed for lupanine isolation from a short number of fractions rich in lupanine. From Fig. 6 it is possible to confirm that lupanine is recovered in the EtOH fractions and that a water washing step is needed for resin regeneration, confirming the results in batch binding experiments. The kinetic experiments (Fig. S4; Table S5) indicated that the system needs about 2 h to reach equilibrium. However, due to practical limitations of experimental set-up, the column assay was performed at a flow of around 2 mL/min, corresponding to a residence time of 50 min, thus quite lower than the 2 h. Consequentially, the results for the column and the batch experiments are different, as summarized in Table 3. Furthermore, it was possible to determine the reusability of this procedure for 6 cycles of binding, recovery and regeneration with values between (91−98)% for lupanine binding, (70−81)% for its recovery in EtOH fractions with a purity around 48%. It was also observed that in a 7th cycle, the binding percentage dropped almost 8 times to 12%, with a recovery of only 41.7% (Table S6).

The use of XAD-16 resin with EtOH recovery is able to isolate lupanine from still significant larger volumes of wastewater nanofiltration retentates and provide a lupanine rich crude with a purity around (52−67)%. However, such concentrated lupanine crude will require further polishing to recover lupanine with a lower percentage of organic matter contamination.

3.4. Solvent extraction experiments

3.4.1. Solvent selection for lupanine extraction from wastewaters
Solvent extraction was assessed as one possible way to isolate lupanine after the resin purification step or directly from the

retentate, rich in lupanine and with high COD content, from the NF experiments. Considering that lupanine is better extracted on its neutral form (pKa = 9.1) the aqueous solutions pH was adjusted to 13−13.5. The solvents showing a higher lupanine extraction efficiency were DCM, toluene, 1-octanol, 1-butanol, EtOAc and MTBE (Fig. S6).

The selection of solvent should primarily consider solvent extraction efficiency, lupanine purity, solvent contamination of aqueous phase and solvent boiling point, envisaging its recyclability (Tables S7−S9) (Alder et al., 2016; Chickos and Acree, 2003; Levet et al., 2016; Smallwood, 1996). Therefore, EtOAc and MTBE are the most suited solvents for lupanine. From these preliminary assays with MTBE it was possible to recover lupanine with a yield of 57.1% and a purity of 70%, while for EtOAc a yield of 84.4% was achieved with a purity of 80% (Fig. S6).

3.4.2. Lupanine extraction with MTBE and EtOAc at larger scale and successive extractions
After solvent screening for lupanine isolation from the wastewater at a small scale, a solvent extraction was performed with 150 mL of cooking phase wastewater using MTBE and EtOAc. In both cases, it was possible to achieve more than 80% of lupanine purity (Table 4) with similar recoveries as observed in the screening assays.

To improve even further lupanine recovery, additional successive extractions of the cooking wastewater were performed. The distribution coefficients of lupanine between the wastewater and MTBE ($K_p = 2.22 \pm 0.50$) or EtOAc ($K_p = 3.57 \pm 0.82$) where estimated. Using a ratio of 1:1 for each single extraction and 4 or 3 successive extractions for MTBE and EtOAc respectively, it was possible to recover (92−98)% of lupanine with a similar purity of around 77% for both solvents. The improved extraction protocols, using higher amounts of solvent than the initial extraction method used, results on higher lupanine recovery yields with small losses on purity; indicating the hydrophilic nature of contaminants. Still, consistent with the higher distribution factor estimated, the results for EtOAc were achieved using less organic solvent. For this reason, EtOAc is the organic solvent suggested to integrate a possible strategy of solvent extraction for lupanine isolation.

3.5. Proposed strategy

In Scheme 2 are presented three main strategies for lupin beans debittering wastewater possible treatment with lupanine isolation for further valorization. All these three strategies consider first a nanofiltration with NF270 membrane to obtain a permeate of the treated water representing about 80% of the debittering step wastewater and a concentrated retentate enriched on lupanine. Lupanine is then isolated from such retentate either by: (1) an adsorption step using XAD-16 resin by column chromatography, (2) multiple solvent extractions with EtOAc or (3) an adsorption step using XAD-16 resin by column chromatography followed by multiple solvent extractions with EtOAc (Scheme 2, Table 5).

In the strategies presented in Scheme 2, around 97% of lupanine and 94% of the organic matter of the sample are retained in the first NF stage, corresponding to a stream where lupanine represents only 19.4% of the total organic matter contributing to COD (Table 6).

In the strategy comprising (NF + CC), the NF stage is followed by an adsorption step with XAD-16 resin by column chromatography. At this point the overall yield for lupanine recovery was around 75%, but the final purity achieved was only around 48%. To further improve the purity of this lupanine fraction, in the strategy comprising (NF + CC + SE), a solvent extraction step with EtOAc was performed only for the fractions of the column which showed the presence of lupanine. After this unit operation the overall yield

Table 4
Lupanine extraction and purity from cooking phase wastewater.

Extraction protocol	AcOEt		MTBE	
	Initial 2 step extraction	Improved 3 step extraction	Initial 2 step extraction	Improved 4 step extraction
Recovery (%)	74.50 ± 0.01	98.40 ± 0.20	40.00 ± 0.01	92.53 ± 0.71
Purity (%)[a]	81.86 ± 1.94	78.41 ± 2.59	85.90 ± 0.01	76.52 ± 1.92

[a] – $COD_{lupanine}/COD_{total}$ after lupanine recovery (%).
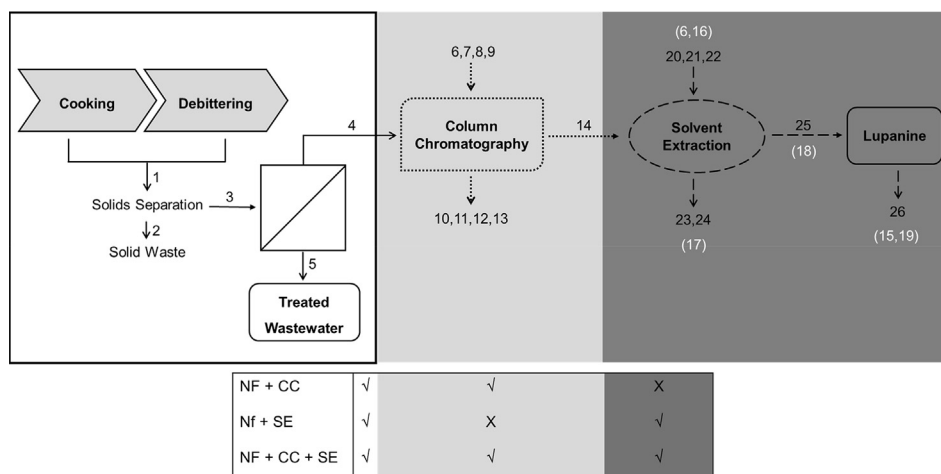
Scheme 2. Proposed strategies for lupin beans industrial wastewater treatment with lupanine isolation.

Table 5
Identification of the streams in proposed strategies for lupin beans wastewater treatment and lupanine isolation.

Stream	Description	NF CC	NF SE	NF CC + SE
1	Cooking and debittering wastewater	$1 m^3$	$1 m^3$	$1 m^3$
2	Solids separated from lupin beans wastewater	2%	2%	2%
3	Aqueous stream obtained after solids separation	$1 m^3$	$1 m^3$	$1 m^3$
4	NF retentate: rich in lupanine and other organic species	$0.23 m^3$	$0.23 m^3$	$0.23 m^3$
5	NF permeate: treated wastewater	$0.77 m^3$	$0.77 m^3$	$0.77 m^3$
6	NaOH for NF retentate pH adjustment	11.5 Kg	11.5 Kg	11.5 Kg
7	Adsorber: Amberlite XAD-16	34.5 Kg	–	34.5 Kg
8	EtOH: for lupanine recovery from XAD-16	$0.14 m^3$	–	$0.14 m^3$
9	Aqueous stream for regeneration of the adsorber	$0.14 m^3$	–	$0.14 m^3$
10	Spent basified water from column adsorption	$0.23 m^3$	–	$0.23 m^3$
11	Spent adsorber (XAD-16)	34.5 Kg	–	34.5 Kg
12	Spent aqueous stream from the adsorber regeneration	$0.14 m^3$	–	$0.14 m^3$
13	Spent EtOH from lupanine recovery	$0.10 m^3$	–	$0.10 m^3$
14	Concentrated organic phase enriched in lupanine (EtOH)	$0.034 m^3$	–	$0.034 m^3$
15	Spent EtOH	$0.034 m^3$	–	–
16	Organic Solvent (EtOAc)	–	$0.69 m^3$	–
17	Spent basified aqueous phase	–	$0.23 m^3$	–
18	Organic phase rich in lupanine	–	$0.69 m^3$	–
19	Spent organic solvent (EtOAc)	–	$0.69 m^3$	–
20	Water for dissolution of dry residue from EtOH	–	–	$0.034 m^3$
21	NaOH for aqueous phase pH adjustment	–	–	0.72 Kg
22	Organic solvent: EtOAc	–	–	$0.10 m^3$
23	Spent EtOH	–	–	$0.034 m^3$
24	Spent aqueous phase	–	–	$0.034 m^3$
25	Organic phase rich in lupanine (EtOAc)	–	–	$0.10 m^3$
26	Spent organic solvent (EtOAc)	–	–	$0.10 m^3$

of the intermediary step was kept constant, around 75%, but it was possible to obtain a purer fraction of lupanine in the end at 78%. A simpler alternative to the previous strategy, would comprise (NF + SE), in which after the NF stage, a multiple solvent extraction step with EtOAc is proposed allowing to obtain an overall yield of 95.4% for lupanine recovery which is around 78% pure.

Comparing the several strategies, it is possible to observe that driven by the performance of the solvent extraction step, both strategies (NF + SE) and (NF + CC + SE) reach the same final purity of around 78% (Table 6), but with higher losses of lupanine in the last one. In strategy (NF + SE) more extractant organic solvent, EtOAc is used than in strategy (NF + CC + SE). However, this

Table 6
Summary of lupanine yield and purity obtained with different strategies for its isolation from lupin beans industrial wastewaters according to strategies exemplified in Scheme 2.

	Lupanine	
Operations	Yield (%)	Purity (%)
NF + CC	75.7	48.0
NF + SE	95.4	78.4
NF + CC + SE	74.4	78.4

NF — Nanofiltration; CC — Column chromatography; SE — Solvent extraction. Yields for isolated operations: 97.0% for NF, 98.4% for SE and 78.0% for CC.

strategy uses also other solvent, EtOH, and additionally to having a lower lupanine yield, is also more cumbersome, requiring more equipment and materials than in (NF + SE). Therefore, the simpler process scheme with a nanofiltration and a solvent extraction is the process route recommended by this study.

A previous study reports osmotic evaporation followed by celite adsorption in basic medium, filtration and successive extraction with petroleum ether, Et_2O and DCM, to recover lupanine from lupin beans wastewater in the Et_2O fraction. In this case an osmotic agent was used, calcium chloride, and the membrane process was run at 32 °C to keep the work flux constant through the time of experiment (Carmali et al., 2010). With this method it was possible to recover only 18.5% of lupanine with 90% purity. In the strategies we propose it is possible to have a higher recovery yield of lupanine between 75% and 95%. Therefore, considering a batch production of 2.5 tons of lupin beans, generating 60 m^3 of debittering wastewater, it is possible to recover (5.36—6.76) Kg of lupanine per ton of dry beans at around 78% purity. Such enriched lupanine crude will require further polishing to recover lupanine with a lower percentage of organic matter contamination. That is possible through further purification of this smaller sample of lupanine using for example recrystallization from hexane. However, to show the feasibility of using lupanine batches with purities around 78%, lupanine conversion into sparteine was assayed.

3.6. Lupanine conversion in sparteine

The lupanine isolated from lupin beans wastewater was reduced to sparteine by applying a reported procedure using $NaBH_4/I_2$ methodology (Maulide et al., 2014), followed by distillation under reduced pressure allowing the isolation of pure (by NMR) sparteine in 60% yield, a value similar to the one obtained using pure lupanine. This result suggests that remaining impurities, carried out with the crude lupanine isolated by solvent extraction from the wastewater, do not affect the chemical reaction performance with the final distillation step allowing their removal efficiently.

4. Conclusions

Several unit operations were assessed separately and optimized for lupin beans wastewater treatment and recycling and lupanine alkaloid recovery.

Importantly, the NF270 membrane showed the highest lupanine rejection (99.5%) originating a lupanine rich retentate.

While the rejection for lupanine was high, the rejection for the other organic matter present in the wastewater was equally high (94%). Therefore, further isolation of lupanine from this stream was evaluated by solvent extraction and resin adsorption. XAD-16 resin was the selected adsorber, leading to 77% of lupanine yield but only with 48% purity by column chromatography.

Notably, EtOAc was the best performing solvent for lupanine recovery, allowing a recovery of 98% of the lupanine, with the highest purity achieved (78%), using three successive extractions. The use of EtOAc, uses less organic solvent when compared to MTBE.

Overall, the best combination for lupanine isolation comprised a NF stage followed by multiple EtOAc solvent extractions, being possible to recover 95% of lupanine with a purity of 78%.

The treated water obtained in the NF permeate, representing 80% of the debittering step wastewater, can be further re-used within the industrial process addressing a major environmental issue concerning savings in the use of high amounts of fresh water in the lupin beans debittering process. The lupanine fraction obtained after NF and successive organic solvent extractions can successfully be used in the process of conversion to sparteine with a final purity above 95%.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2020.123349.

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