# Towards the development of a process for lupin beans detoxification wastewater with lupanine recovery

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**Abstract** Every year, large amounts of fresh water is used by food processing industry to deliver safe food to society. The resulting high volumes of wastewater have to be treated before released back to the environment. The present work considers lupin beans processing wastewater and discusses effectiveness of approaches for water treatment, able to recover lupanine, an alkaloid with pharmaceutical properties. Nanofiltration, ion exchange, liquid-liquid extraction are among the separation units evaluated for lupanine wastewater detoxification, with lupanine rejection, binding or extraction higher than 99% when using NF270 nanofiltration membrane, a strong acidic resin (e.g. Amberlyst36) and an extracting solvent, respectively. A process for lupin beans wastewater detoxification and lupanine recovery was envisaged with three sequential operations: a centrifugation for removal of suspended solids, a nanofiltration operation using a NF270 membrane to obtain a detoxified water permeate and a lupanine rich retentate, and a solvent extraction of the retentate stream for isolation of lupanine from aqueous soluble compounds. Alternatively, an additional lupanine purification stage using resin Amberlyst36 is also considered, between the nanofiltration and solvent extraction for additional purification and volume reduction. Additional work-up would include solvent exchange by distillation of extraction solvent and addition of hexane to promote lupanine crystals formation to be isolated by decantation. The envisaged process was modelled using the SuperPro Designer on the basis of the detoxification of wastewater from selected fractions with higher lupanine contents resulting into the estimation of lupanine recovery higher than 98.5% for an optimistic scenario and with a purity >95%.

**Keywords:** Lupanine, Alkaloid, Detoxification, Nanofiltration, Resin, Liquid-Liquid Extraction, Amberlyst36, NF270, SuperPro Designer.

## 1. Introduction

Nowadays with the increasing population numbers, it is important to find and increase food sources that can sustain the high nutritional needs of people. Lupine is undoubtedly an option to consider due to its high nutritional value , with high protein (32-52%) and low unsaturated fat (>20%) content[1,2]. Each year in Europe, approximately 500,000 tons of goods containing lupins are consumed, including the traditional snacks.[3] However, lupin seeds contain quinolizidine alkaloids, QA, (2%) in their composition, mostly Lupanine, Sparteine, Lupinine and multiflorine though the quantity and variety of those alkaloids is highly variable between species. QA are toxic and have bitter taste which renders the seeds improper for consumption without prior treatment.[2,5].The Lupanine, although toxic, also have beneficial properties for human health such as antidiabetic properties, Antiarrhythmic agent, biological plant protector and as a starting material for the semi synthesis of other alkaloids.[6,7,8,9,10,11] The extensive debittering that is need to eliminate the concentration of alkaloids to the value of alkaloid content marketable sweet of 130-150 mg/kg), leads to high volumes of wastewater every year. Thus, it is mandatory alternatives to save water in this process. In this work is given insights to reduce the amount of water used for lupin beans debittering process and the preliminary studies to develop a route for detoxification of the wastewater generated in that process; allowing

water with quality enough for being recycling in the process, while recovering lupanine.

To achieve the aim of this thesis several step objectives were defined: (i) The industrial lupin bean debittering process wastewaters, where the lupanine can be found, was characterized in order to understand in which phases of the water treatment process the toxic lupanine is at higher concentrations. (ii) Different unit operations were studied to choose the best method for water treatment and recovery of lupanine. Permselectivity of different ultrafiltration and nanofiltration membranes (NADIR010 and 030, NF90, 200 and 270); lupanine binding to different resins (Amberlyst15 (wet), 16 (wet), 36; Amberlite IRC50, IRC86, IRA68, IRA458, AG50W-X2; XAD7, and CG-400) was evaluate and different regeneration protocols assessed; and coefficient of extraction of lupanine from an alkaline solution for different solvents (dichloromethane, toluene, hexane, heptane, methyl t-butyl ethane, methyl isobutyl ketone).(iii) Finally, it was also included a theoretical chapter of the process, where SuperPro Designer 8.5 was used to model potential processes for wastewater detoxification and lupanine isolation and assessing the efficiency of the project.

### 1.1. Lupanine

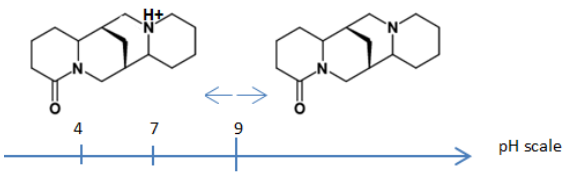


Figure 1 -Protonate and not-protonate Lupanine throughout the pH range.

Lupanine is a tetracyclic quinolizidine alkaloid toxic for human and animal health. Alkaloids form free bases (deprotonated) in alkaline solutions and they are usually insoluble in water but soluble in organic solvents. On the other hand, at acidic conditions alkaloids are usually soluble in water but insoluble in organic solvents. This different solubility is very important to isolate and purify alkaloids.

## 2. Results and Discussion

### 2.1 Characterization of industrial wastewater from lupin beans debittering process

A case study of lupin bean industrial process (Case study A) is considered in this thesis and represented in Figure 2. The process comprises 4 stages: (i) In the hydration stage, $M_{dry\_beans}$ ton of dry lupin beans and V0 m3 of water are loaded in a $V_{Total}$ m3 tank (hydration tank) and left to swell for $T_{hidratation}$. The swelling mass have ratio of 1.8 $M_{swollen\_beans}/M_{dry\_beans}$, and 784 kg of water intake per ton of dry beans. (ii) In the cooking phase, $M_{hydratate\_beans}$ ton of swollen beans are submitted at $TEMP_{boiling}$ ºC for $T_{cooking}$ minutes in a boiler to allow lupanine to be extracted through the beans walls. (iii) In the swelling process, swollen beans and cooking water are transfer from boiler to another tank (Detoxification Tank) where they are cooled down to room temperature and left in contact for several days ($T_{Min\_Swelling}$ to $T_{Max\_Swelling}$ day). By the end of the swelling process, the lupin beans are left inside the tank, but the water is discharged, yielding a $V_{High}$ m3 of a wastewater stream labelled as “High”, as it is the stream with the highest concentration of lupanine from the whole extraction process, namely $C_{High}$ = 3.4 g/L (iv) In the sweetening stage the Detoxification Tank with the $M_{hydratate\_beans}$ ton of beans is refilled with clean water with a V1 m3 of water (=$V_{High}$). In Case study A: the

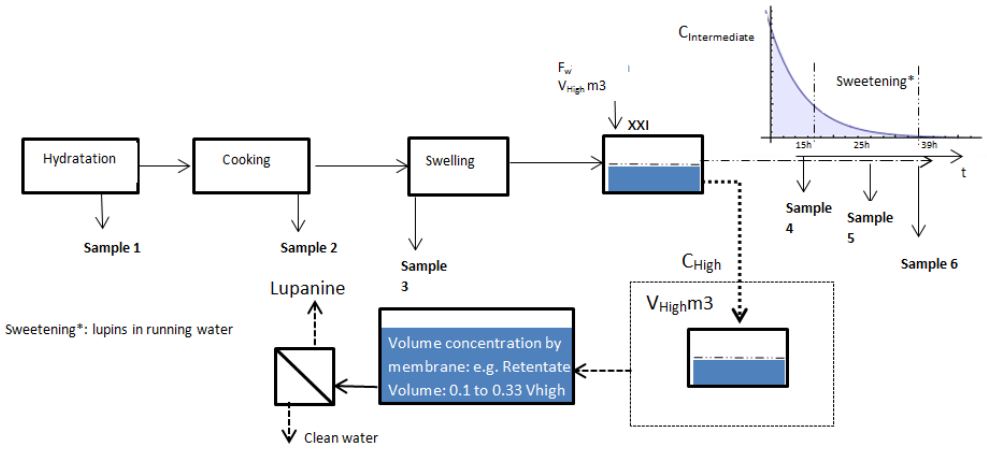


Figure 2 -Process scheme of one lupin bean industry A.

sweetening is carried out in a continuous mode, fresh water is added to the tank at a flow of $F_{sweetening}$ (or Fw) m3/h for $T_{sweetening}$ hours, the volume of solution in the tank is kept constant over such time as the outflow of wastewater continuously discharged equals the inflow of fresh water added. The total volume of wastewater discharged $V_{sweetening}$ will have different lupanine concentrations, starting from a higher value to a value in which virtually all the lupanine has been extracted from the beans and the concentration of lupanine in beans and water is negligible. Therefore, the wastewater volume resulting from the sweeting stage will be subcategorized in $V_{Medium}$, $V_{Lower}$ and $V_{Negligible}$ according with decreasing lupanine concentrations. Diavolumes are inhere defined as $Di = \frac{Fw}{Vw} T^{i}_{sweetening}$.

In an initial characterization of the of the wastewater generated in the process the collected wastewater from the extraction process for the continuous case study A as:

Phase 1: from hydration process; Phase 2: from the cooking water; Phase 3: from swelling process; Phase 4: a sample at 38.5% of total sweetening time (38.5%$T_{sweetening}$); Phase 5: a sample at 64.1% of total sweetening time (64.1%$T_{sweetening}$) and Phase 6: a sample at the end of total sweetening stage (100%$T_{sweetening}$).

For each water phase, the lupanine concentration was measured, as well as the chemical oxygen demand (COD), total reducing sugars, total protein, total organic carbon (TOC), dry matter, pH, conductivity and viscosity. In Table 1 is the summary of the results of the different tests made to the water of the different phases and considering the results it is possible to confirm that the water from phase 3 is the richest when compared to the others. For this reason, the water from phase 3 was chosen for the subsequent experiments.

To further analyze this process, water samples from the sweetening phase were collected every 10 minutes for times below $T_{sweetening}$ using a peristaltic pump. The water composition was analyzed and with the obtained data, a concentration profile was made over time. The results clearly show that the lupanine concentration reaches a value below the detection limit of the analytical method (at a lupanine concentration of 1.95 mg/L) at a washing time of approximately 38.5%$T_{sweetening}$, meaning that the concentration of lupanine in the water is nearly zero, and from this point on, it may not need

further treatment. This result would indicate that the amount of water needed to extract the total lupanine content from the lupin beans in this run would in fact be less than the one currently used.

The concentration profile of lupanine in a tank of volume V can be described by writing a component material balance over the solute as indicated below:

$$V\frac{dC}{dt} = F_{wi}C_{add} - F_{w1}C_t \qquad \text{(Equation 1)}$$

where $C_{Add}$ and $C_t$ (g/L) represent the concentration inlet and outlet of the stream, respectively, and $C_1$ is the average concentration in the tank. Vw (L) is the working volume of liquid solution in the tank, which is maintained constant; and Fwi, Fw1 (L/h) is the flow rates of fresh water added to and wastewater leaving the tank, respectively, which are equal to each other (Fw = Fwi = Fw1) and also constant in time. Since fresh water is added to the system, $C_{add}$ = 0 g/L the equation can be rearranged and integrated, as follows:

Table 1 -Water samples characterization. *distilled water viscosity=1.12 cP; conductivity=1.93 µS. **analysis made by IST Water Analysis Laboratory.

| Process phases | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Lupanine (g/L) | 0.000±0.000 | 1.674±0.009 | 3.444±0.019 | 0.943±0.003 | 0.468±0.001 | 0.045±0.002 |
| Total reducing sugar (g/L) | 0.07±0.00 | 0.59±0.01 | 0.82±0.02 | 0.16±0.00 | 0.11±0.01 | 0.07±0.01 |
| Total protein (g/L) | 0.00±0.03 | 0.107±0.030 | 0.323±0.109 | 0.029±0.021 | 0.026±0.030 | 0.032±0.030 |
| COD (g($O_2$)/L) ** | 0.005 | 23.0 | 30.0 | 8.5 | 4.7 | 1.0 |
| TOC (gC/L) ** | 0.0023 | 8.6 | 15.0 | 3.6 | 1.9 | 0.4 |
| Dry matter (g/L) | 0.48 | 26.20 | 29.55 | 7.93 | 4.27 | 1.12 |
| pH | 7.13 | 5.70 | 3.89 | 3.81 | 3.80 | 3.96 |
| Conductivity (mS) | 0.671 | 3.92 | 7.64 | 2.71 | 0.135 | 0.625 |
| Viscosity (cP) | 1.57 | 1.14 | 1.33 | 1.24 | 1.10 | 1.30 |

$$Vw\frac{dC(t)}{C_t} = -F_w dt \Leftrightarrow \int_{C_0}^{C_t}\frac{dC(t)}{C(t)} = -\frac{F_w}{Vw}\int_0^t dt$$

$$\Leftrightarrow ln\frac{Ct}{C_0} = -\frac{F_w}{Vw}t \Leftrightarrow$$

$$Ct = C_0 exp^{-(\frac{F_w}{Vw}t)} \qquad \text{(Equation 2)}$$

where $\frac{F_w}{V}$ is the dilution rate, D ($h^{-1}$) and $C_0$ is the lupanine concentration in the starting of the sweetening stage. Integrating equation 2 and multiplying Fw, it is possible to calculate the amount of lupanine in the wastewater collected until the maximum integrating time: $M^t_{Sweetening} = Fw\int_{C0}^{C(t)} C(t).dt$

$$= Vw.C0.\left[1 - exp\left(-\frac{F_w}{Vw}t_{Sweetening}\right)\right]$$

(Equation 3)

To calculate the concentration of a stream collected from the beginning of the sweetening stage to a time t, one need to consider the volume collected of such stream as D.$V_w$:

$$C^{comulative\ to\ t}_{Sweetening} = \frac{M^t_{Sweetening}}{D.Vw} = \frac{C0}{D}.[1 - exp(-D)]$$

(Equation 4)

Therefore, to estimate the % of lupanine already eluded already in the wastewater for each diavolume one could theoretically use the expression:

$$\%M^t_{Sweetening} = \frac{M^t_{Sweetening}}{M^{total}_{Sweetening}} = 1 - exp(-D)$$

(Equation 5)

Using such equation one can calculate for different conditions how many diavolumes are needed to achieve a given lupanine removal efficiency as illustrated in the Figure 3 where a 75%, 85% or 90% of lupanine recovery was aimed, one would need a diavolume of about 1.4, 1.9 or 2.4.

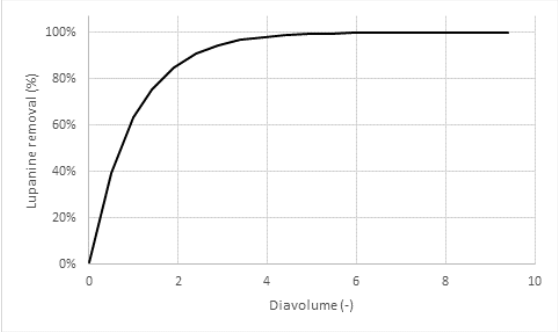


Figure 3 -Percentage of lupanine recovery when adding x times the volume of water being treated

## 2.2 Unitary Operations and process design

To study different routes for detoxification of this wastewater and isolation of lupanine it was used different unit operations that are next going to be discuss. At the center of the process envisaged it is a nanofiltration (NF) membrane able to provide clean water in the permeate and a lupanine concentrate retentate. Upstream of the NF it is envisage operations that contribute to removal of macromolecules and other fouling agents, such as a centrifugation followed by an ultrafiltration (UF). The solids isolated from the centrifugation and the retentate from the UF could be used to make methane, which could be transformed into energy to warm up the water where the beans are boiled, for example. After extracting the bigger molecules, the water would pass through a NF system where the membranes with a molecular cut off lower than the molecular size of lupanine so that the lupanine would be retained and from the permeate would leave clean water. This operation would also allowed a reduction of the water volume. Downstream the NF stage, the retentate could be sent to further purification. Having as option the use of a resin, which would bind the lupanine while allowing elution of other impurities followed by recovery of the toxic with eluent; or a solvent extraction for lupanine recovery.

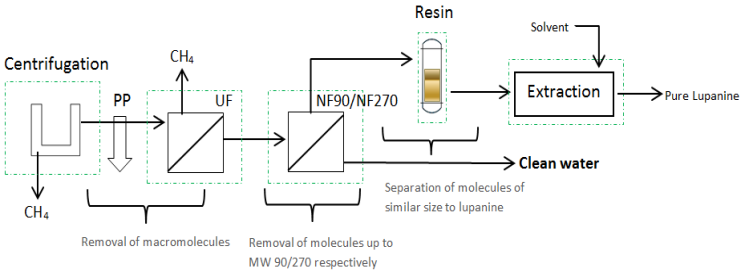


Figure 4 -Process diagram flow of a lupin bean wastewater treatment in industry. Starting from the left to the right: centrifugation, ultrafiltration, nanofiltration, resin and extraction operations are represented

### 2.2.1 Nanofiltration and Ultrafiltration

The membranes studied in this work were the ultrafiltration membranes, NADIR010 and NADIR030 and the nanofiltration membranes NF90, NF200 and NF270 at 10bar and 24bar respectively. The membranes were studied on their flux, flow, permeability and rejections for COD, total reducing sugars and lupanine.

The ultrafiltration membranes had a lupanine rejection higher than expected and a rejection for COD significantly lower, considering that one expects a large contribution of macromolecules for COD. It was hypothesize that 10 bar pressure was too high for this membranes affecting the results. However, the use of 5 bar pressure didn't improve membrane selectivity. The aim of using nanofiltration membranes is to retain the lupanine in the retentate, while providing a permeate of clean water. As shown by the results, all the membranes reject very well the lupanine (rejections above 99% in all cases) as well as having a good performance in retaining COD and the total reducing sugars. Comparing

all the nanofiltration membranes, the ones with higher rejections for lupanine are the NF270 and NF90, with the former having a slightly higher rejection value, as it is possible to see in Figure 5.

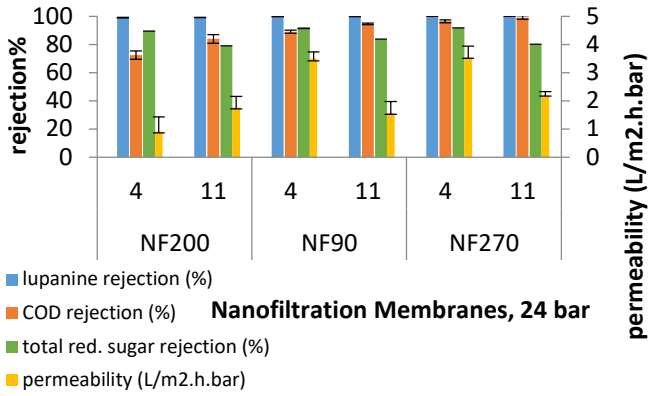


Figure 5 -Lupanine, COD and total reducing sugar rejections in the primary axis; permeability in the second axis for nanofiltration membranes at 24 bar.

### 2.2.2 Ion Exchange Resins

The water of the retentate would contain lupanine and many other organic and inorganic molecules of similar or larger size that would be of interest to separate from lupanine. The next step was the study of the various resins (strong acidic resins:AG50W-X2, Amberlyst 15,16 and 36 and weak carboxylic resins: Amberlite IRC 50 and 86) for binding lupanine from an aqueous alkaline (pH around 9) solution, an acidic water from phase 3 (pH around 4) and a NF90 membrane retentate (pH 4). Four experimental conditions were tested:(i) In the first condition for lupanine binding by the resins was tested in water without further pH adjustment (pH 9), and (ii) In a second trial was performed with an aqueous solution of lupanine that had been submitted to $CO_2$ bubbling to decrease the pH to near of 4 (the same pH as that of the water from phase 3). However, after seven hours of bubbling, the pH still decreased to 6. While all the weak and strong resins present high binding efficiencies in water solutions at pH 9 (pH was not adjusted), weak acid resins show lower performance for solutions at pH 6, where pH was adjusted. The results for the two other conditions for lupanine binding to this set of weak and strong acidic resins are presented in Figure 6 and use real effluents the use of lupanine rich (iii) wastewater from phase 3 and (iv) the NF90 retentate.

From Figure 6 it is possible to see that the weak carboxylic resins are more influenced by the pH than the sulfonic ones. For the retentate stream, with high concentration of lupanine, as well as other species concentrated in the nanofiltration process, the binding percentage of lupanine were lower. The reason could be due to resin saturation (either by lupanine or other species).

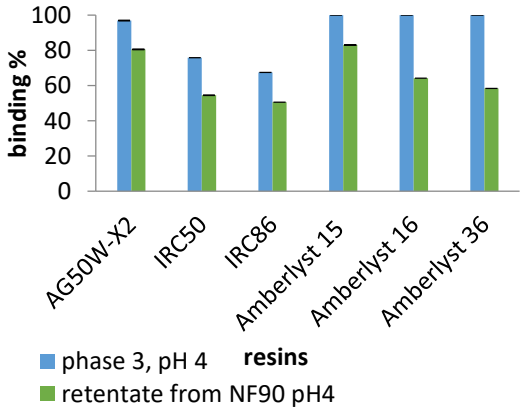


Figure 6- Binding of the lupanine with the different acidic resins for the phase 3 and for NF90 retentate, at pH 4.

### 2.2.3 Solvent Extraction

Solvent extraction of lupanine in an organic solvents from a concentrated aqueous phase allows further concentration of this compound, while separating it from water soluble compounds and isolating lupanine in a stream easier to process chemically or further concentrate by solvent distillation. The selected (Hexane, Heptane, Toluene, Methylisobutyl ketone, Dichloromethane, t-

Butylmethyl ether) solvents for the extraction process had to respect the following requirements: (i) allow the formation of two phases with water, (ii) not being hydrolysis by NaOH, (iii) efficient lupanine extraction and (iv) volatility (v) sustainable and environmental friendly. Considering the water from phase 3 of the lupanine extraction process, two extractions were made with each solvent in a proportion of 2:1, twice. The results are shown in Figure 7.

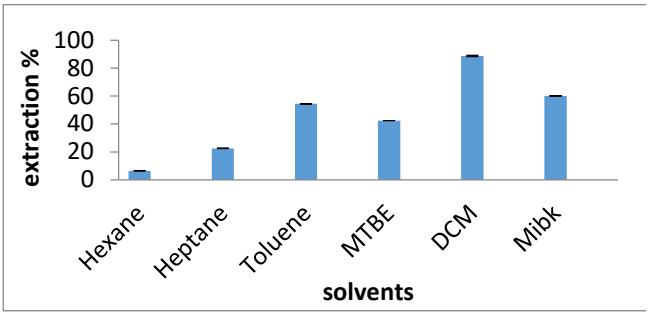


Figure 7 - Lupanine extraction for different solvents.

According to the Figure 7 the solvent with the best extraction result was DCM.

### 2.3 SuperPro Design

Two different process schemes are inhere considered for modelling. SuperPro Designer v8.5 software was used. Considering the initial process envisaged, the options modelled do not include the use of an ultrafiltration, instead, upstream to the nanofiltration only a centrifugation is considered. Downstream the nanofiltration, both processes comprise a solvent extraction. In addition to the operations experimentally studied in this thesis, it is also the modelling of a solvent exchange of the lupanine from the extracting solvent to hexane, with distillation of the former and addition of the later solvent. Lupanine can be isolated from hexane in crystals.

The difference between process scheme 1 and 2 is that in process scheme 2, between the nanofiltration and the solvent extraction, it is also include a resin binding step and the respective regeneration stage. Since the regeneration uses an ethanol/water solution, an additional solvent distillation is needed to remove such solvent. In both process schemes, only a fraction of the wastewater generated in the lupin beans debittering process is targeted. Specifically, the fractions with higher lupanine concentration comprising a total volume of 2.54 Vw. This fraction comprises about 85% of the total lupanine on the debittering wastewaters at a composite concentration of about 2 g/L lupanine. According with the simulations performed the process schemes envisaged allows for 98.5% and 97.4% recovery of the lupanine in such wastewater fractions considered the first and second option scheme suggested.

## 3. Material and Methods

### 3.1 Lupanine quantification

Lupanine was quantified by HPLC Merck HITACHI, La Chrom (UV Detector L-7400; Interface D-7000, Programmable Autosampler L-7250) and both the column and the pre-column were from Kinetex (5µm EVO C18 100Å, 250x4.6 mm). The solvents used were: A – 0.01 M phosphate buffer ($Na_2HPO_4$, pH 10.5) and B – Acetonitrile (MeCN) with a flux of 1 mL/min with 85% A / 15 % B for 25 minutes, an injection volume of 20 uL, and detection at 220 nm. To prevent salt precipitation, the equipment was washed with milliQ water after the injections and the HPLC column was stored in a mixture of 10 % milliQ water and 90 % MeCN. The injections were performed at room temperature. *The samples before injected were prepared in the following method:* about 18.3-35mg/mL of KOH were added to 5mL of the sample to reach a pH

value between 13 and 13.5. The sample was centrifuged for 2 min (2000G, IKA) and the supernatant was filtered a syringe filter (syringe filter 0.20µm; 13mm; Nylon, Tecnocroma). The membrane filters used to prepare aqueous HPLC solvents (water milliQ and 0,01M $Na_2HPO_4$ buffer from Panreac) were from Whatman, Schleicher&Schuell, mixed cellulose ester (diameter=47mm, Pore size=0.45µm). Acetonitrile used was HPLC grade(Mw:41.04, CAS: 75-05-8) from Fisher Chemical; Disodium phosphate ( Mw:141.96G/mol) was from ,Panreac; Potassium hydroxide Mw:56.11g/mol (Panreac, CAS:1310-58-3); Potassium phosphate tribasic monohydrate (Sigma-Aldrich, CAS: 27176-10-9).

### 3.2 Nanofiltration

The water from the phase three was subjected to filtration. Those processes were performed by using nanofiltration membranes (Dow FILMTEC Membranes NF90, NF200, NF270) and ultrafiltration membranes (Lenntech, MICRODYN-NADIR NP030 P; NP010 P) also using a pump gear from ISMATEC BVP-Z.

In these studies, water from phase 3 collected in *Tremoçeira,* without pre-treatment, was used. 400mL were centrifuged at 6000rpm for 30min at 20°C. The supernatant from the centrifugation was then subjected to a nanofiltration and ultrafiltration, using the selected membranes (NF90, NF200, NADIR010 and NADIR030) from Filmtech, in "cross flow" mode at room temperature, with recirculating 350 cu. The NADIR membranes were operated under an applied pressure of 10 bar while the NF membranes were operated at 24 bar. 50 ml of the supernatant were separated and labelled as "Original"; 350 ml of the supernatant were transferred to the nanofiltration system, homogenized by recirculating the solution (without the application of pressure) for 5 min and, subsequently, an aliquot was collected and tagged as "Feed". The pressure applied to the membrane was to promote the solution's nanofiltration through both membranes in series, placed in the cells A and B. The flow through the membrane as well as the membrane's permeability were quantified. By the end of the nanofiltration, the solutions that passed through both membranes A and B were collected and labelled as "Permeate" whereas the solutions that got retained were labelled as "Retentate". The conductivities of the "Original", "Feed", "Permeate" and "Retentate" solutions were then measured.

### 3.3 Ion Exchange Resins

In order to assess the resin with higher lupanine binding and recovery, different resins were studied: Amberlite IRC86, Fluka, CAS: 211811-37-9; Amberlite IRC50, Rohm and HAAS france S.A; Amberlite AG50W-X2, Bio-Rad Laboratories; Amberlite IRA 68, Rohm and HAAS france S.A; Amberlite IRA 458, Rohm and HAAS france S.A; Amberlite resin CG-400, laboratory BDH reagentAmberlyst 16 (wet), Fluka, CAS:125004-35-5; Amberlyst 36, Sigma-Aldrich, CAS:039389-20-3; Amberlyst 15 (wet), Sigma-Aldrich, CAS: 39389-20-3; Amberlyst 16 (wet), Fluka, CAS:1250004-35-5; XAD7, Rohm and HAAS france S.A. The solvents used to study the resin regeneration process were: Ethanol (Merck ACS., CAS No. 64-17-5), NaOH (Eka Pellets, CAS:1310-73-2), HCl (Merck,1.19 g/cm3 (20 °C)). The samples were agitated in a stir plate from AccuPlate Stirred (Labnet). The lupanine binding experiments proceeded as follows:0.15 g of

resin, 1.5 mL of sample and a magnetic agitator were added to an Eppendorf tube of 2 mL, which was then placed in a stir plate at 250 rpm for 15 hours at room temperature; after that, the Eppendorf tube was centrifuged at 1400 rpm for 2 minutes; finally, the supernatant solution was recovered with the help of a needle and a syringe, filtered with a syringe filter and further analyzed in HPLC. And the regeneration of resin as follows: after removing the supernatant of the lupanine binding experiment, the resin was regenerated by adding 1.5 mL of regenerating solution (HCl 10% in water, for example) to the Eppendorf. The same procedure as before was followed, the Eppendorf tube was placed in a stir plate at 250 rpm for 15 hours at room temperature and then it was centrifuged at 1400 rpm for 2 min. The regeneration solution was removed with the help of a syringe and needle to be further analyzed in HPLC.

### 3.4 Solvent Extraction

In this work, the liquid–liquid extraction, or solvent extraction, consisted in transferring the lupanine in the water from phase 3 to another immiscible liquid, in this case an organic solvent. The formation of two immiscible phases is due to the relative solubilities of the liquids. 6 mL of the phase 3 and 3 mL of an organic solvent were added to a test tube, which was closed and agitated in a vortex; two phases were formed upon agitation and the aqueous phase was extracted to a second tube with a Pasteur pipette. From the aqueous phase, 2 mL were collected for HPLC analysis whereas the other 4 mL were subjected to a second extraction; In the second extraction 2 mL of the organic solvent were added to the 4 mL of aqueous phase; the mixture was vortexed and the aqueous phase was recovered for HPLC analysis. The solvents used for this experience were DCM, MTBE, toluene, hexane, heptane, and MIBK. The aqueous phases were injected into the HPLC system after being basified with KOH to pH 13-13.5, centrifuged and filtrated. During the extraction of lupanine it was used: activated carbon from Fagron; celite- Fisher Scientific, ACROS Organics; dichlotomethane- Sigma-Aldrich, CAS: 75-09-2; diethyl ether- density:0.706 g/mL, Mw:74.12 g/mol, bp. 34.6°C; Hexane- Sigma-Aldrich, CAS: 110-54-3; Magnesium sulfate Anhydrous- Fisher Scientific; Sodium hydroxide, CAS:1310-73-2; and lupin beans from the Sociedade agrícola de Alicante, SOCOMAL, Temuco, provincia de cautin-chile.

## 4 Conclusion

The present study allowed the decision of which operations should be performed in the treatment of wastewater from a lupin bean industry, as well as the best methods to recover lupanine from the effluent. Besides the experimental results, an environmental and healthy point of view were taken into account and, of course, it was also possible to assessing the efficiency of the process scheme using the SuperPro Designer. The process scheme of the wastewater treatment would start with a centrifugation, which does not eliminate any lupanine from the water but, on the other hand, eliminates some of the larger molecules reducing the fouling in the following steps. After that, the water would pass through a NF270 nanofiltration membrane (>99% lupanine retention) from where clean water would leave the system. To the retentate it would then be added NaOH to basify the effluent promote efficient extraction of the neutral lupanine to extraction solvent.

Following this process, the solvent would have to be evaporated so that hexane would be added as well and then decanted.

The difference between process scheme 1 and 2 is that in process scheme 2, between the nanofiltration and the solvent extraction, it is also includes a resin binding step and the respective regeneration stage. According with the simulations performed the process schemes envisaged have a potential in an optimistic scenario to reach 98.5% and 97.4% recovery of the lupanine for the first and second process scheme respectively, assuming 99.85% efficiency for lupanine nanofiltration lupanine rejection and resin steps and 99.9% for organic solvent extraction. For a more pessimistic scenario with 90% efficiency for lupanine nanofiltration rejection and resin steps and 90% for organic solvent extraction and a recrystallization efficiency more than half of the lupanine would still be recovered for both process schemes.

Also, according to the Gantt Chart, the option with evaporation takes less time (approximately 25h30) than the procedure using resin (approximately 42h).

Additionally, it would be a good idea to try other ultrafiltration membranes since the one described In this work did not led to good results, as this method allowed the lupanine to pass through the membranes, retaining larger molecules such as proteins. Regarding the lupanine extraction, it would be advantageous to reduce the steps of purification of it as well as reducing the solvents used in the process. As a starting material for the semi-synthesis of other alkaloids for the pharmaceutical industry, it would also be interesting to optimize the extraction of lupanine using enantiomeric separation, instead of performing the separation in the racemic mixture.

## 5. References


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