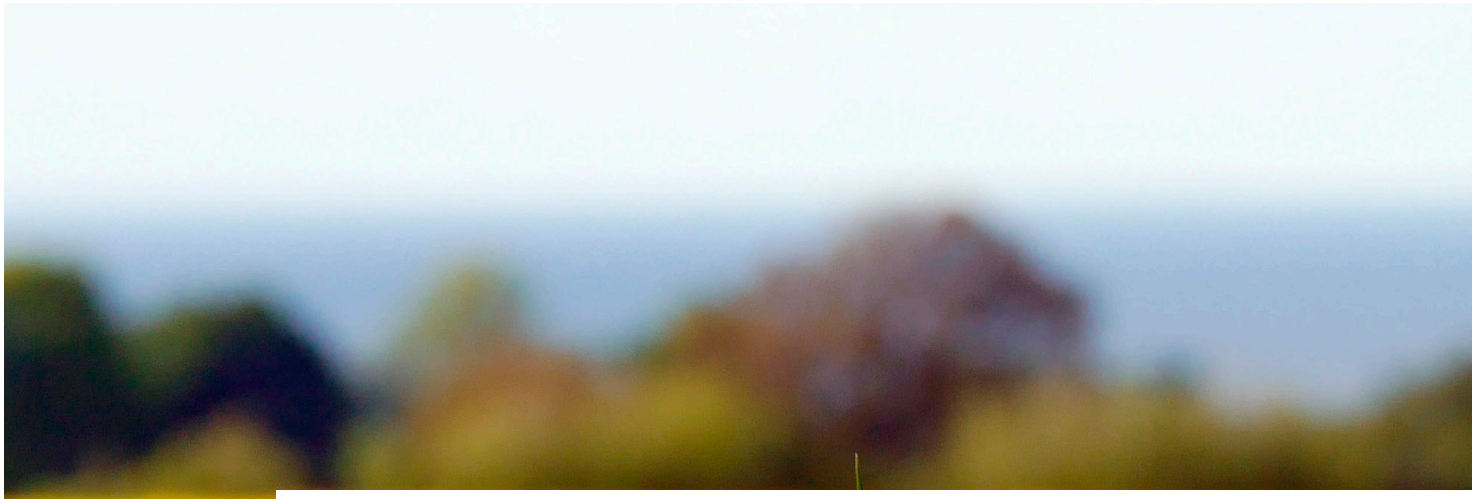

Optimization of Protein Recovery from Rapeseed Press Cake

DEPARTMENT OF FOOD TECHNOLOGY, ENGINEERING AND NUTRITION | LUND UNIVERSITY
CECILIA AHLSTRÖM | MASTER THESIS IN FOOD ENGINEERING 2019

Optimization of Protein Recovery from Rapeseed Press Cake

-

A study of the protein distribution in the various process streams and an investigation whether recirculation of the heavy phase is possible for increasing the protein recovery yield

---

Cecilia Ahlström
2019-01-25

Supervisor: Dr Karolina Östbring

Examiner: Prof Marilyn Rayner

LUND UNIVERSITY

LTH

FACULTY OF ENGINEERING

Abstract

Due to the world's growing population and an increase in food demand, new innovative food products need to be developed to maximize the use of limited farmland. Because of global warming and the fact that plant based products generate less greenhouse gases than meat based products, research should focus on new plant-based protein sources. Rapeseed press cake is a residue from rapeseed oil production and contains around 30 % protein. These proteins can be extracted and possibly be used for food production. However, due to a high concentration of anti-nutrients, the press cake is today mainly sold as animal feed. Previous studies and master students from the Department of Food Technology, Engineering and Nutrition have developed a method for protein recovery from cold-pressed rapeseed press cake including a leaching step and a precipitation step. This master thesis focused on two areas: creating a mass balance of the protein distribution in the existing process and to investigate whether the protein yield could be increased by serial recirculation of unextracted proteins.

The result of the protein distribution showed that the majority of the proteins the first day (52 %) was located in the final sediment as desired, resulting in a protein yield of 52 %. Protein losses in the process was proteins that was never solubilized during the leaching step and therefore remained in the heavy phase (26 %) and proteins that would not precipitate at pH [redacted] remained in the supernatant (18 %). Recirculation of the unextracted proteins resulted in that additionally 9.3 % of the proteins could be collected as sediment increasing the yield to 61 %. Further recirculations only achieved an additional yield increase of 1.0 % and 0.18 % and were not considered economically efficient. The present work shows that recirculation of the unextracted protein should be to perform once.

Populärvetenskaplig sammanfattning

Världens befolkning växer för var dag som går och därmed ökar även behovet av mat. Eftersom arealen odlingsbar mark är begränsad måste vi bli smartare i användandet av åkermark för att kunna föda fler människor. På grund av global uppvärmning och det faktum att växtbaserade livsmedel genererar mindre utsläpp än kött, borde fler resurser fokusera på att utveckla växtbaserade produkter. I dagsläget finns det så kallade sidoströmmar i livsmedelsindustrin som inte används till sin fulla potential. En sidoström är oftast en biprodukt som bildas vid framställning av en annan produkt. Rapsfrökaka är ett exempel på detta. Då rapsolja framställs så pressas rapsfrön och oljan utvinns. Det som blir kvar av fröna efter pressningen kallas rapsfrökaka och innehåller ungefär 30 % protein. Frökakan används i dagsläget till djurfoder trots att dess proteinkvalité är hög och jämförbar med soja. Proteinerna kan nämligen utvinnas och användas till formulering av nya livsmedelsprodukter. En anledning till varför rapsfrökakan inte används i dagsläget är på grund av anti-nutrienter. En anti-nutrient är en molekyl eller substans som minskar upptaget av andra näringsämnen i maten.

Tidigare forskning på institutionen för Livsmedelsteknik på Lunds Tekniska Högskola har tagit fram en metod för att utvinna proteiner från kallpressad rapsfrökaka. Metoden börjar med att frökakan mals för att proteinerna ska kunna ha en chans att lämna frökakan. Den malda frökakan späds sedan ut i vatten. pH höjs och blandningen lämnas på omrörning så att proteiner ska ta sig från frökakan till vattnet istället. Med hjälp av en dekanter separeras sedan frörester från proteinerna. En dekanter är en kontinuerlig centrifug som delar upp material beroende på hur tunga komponenterna är. Ut från dekantern kommer två strömmar, en tung fas som innehåller fröresterna och proteiner som inte velat lämna fröresterna samt en flytande fas som innehåller vatten och majoriteten av proteinerna. Sedan tillsätts syra till den flytande fasen för att neutralisera proteinernas laddning. När nettoladdningen är noll kan proteinerna komma nära varandra och sjunka mot botten. Med hjälp av en centrifug separeras nu lösningen återigen till två faser, en flytande fas som kallas supernatant och en fast fas som kallas sediment vilket även är slutprodukten. Majoriteten av proteinerna återfinns i sedimentet. Syftet med examensarbete var att skapa en massbalans över den existerande processen för att se hur proteinerna fördelar sig i de olika strömmarna under processens gång. Samt att även undersöka huruvida proteinutbytet ökar om man återcirkulerar proteinerna som inte blev utvunnet under processens gång.

Efter att ha genomfört processen en gång analyserades frökakan och de fyra faserna från dekantern och centrifugen. Det visade sig att 26 % av proteinerna fortfarande var kvar i den tunga fasen från dekantern. Därför återcirkulerades den tunga fasen som startmaterial och processen upprepades tre gånger. Totalt tog experimentet fyra dagar och för varje dag analyserades proteinhalten i de fyra faserna.

Resultatet av massbalansen visade att majoriteten av proteinerna (52 %) kunde lokaliseras i sedimentet vilket resulterade i ett proteinutbyte på 52 %. Resterande proteiner lokaliserades i den tunga fasen från första separationen (26 %), i supernatanten då vissa proteiner inte fälldes vid pH [redacted] (18 %) eller fastnade i dekantern under processens gång (4 %). När den tunga fasen återcirkulerades en gång kunde utbytet öka med 9,3 % och därmed höja det totala utbytet till 61 %. Ytterligare cirkulering resulterade endast i en ökning med 1.0 % och 0.18 % och kan därmed inte räknas som ekonomiskt försvarbart. Detta examensarbete visar därmed att återcirkulering av proteiner som inte blivit utvunna ur utgångsmaterialet bör utföras en gång.

Table of Content

1. Background

1.1 Introduction

The global population is growing every day and the food demand increases. Global warming is also a growing problem and is mainly caused by human activity. The earth can only provide a limited amount of farmland so innovative and sustainable ways to produce food needs to be developed. The agricultural sector today are responsible for around 30 % of all emissions of greenhouse gases (GHG) (MacRae, Cuddeford et al. 2013). Animal products generate significantly more GHG than plant-based products. The reasons are that the protein conversion from feed to muscle is low (Figure 1, adapted from Shepon et al) and that ruminants generates methane through their digestion, a GHG more potent than carbon dioxide (Shepon, Eshel et al. 2016). Therefore, there is a need to develop new plant-based protein products to be able to reduce the climate impact of the agricultural sector caused by GHG emissions (Shepon, Eshel et al. 2016). One such strategy could be to investigate by-streams in the agricultural sector that are not used to their full potential.

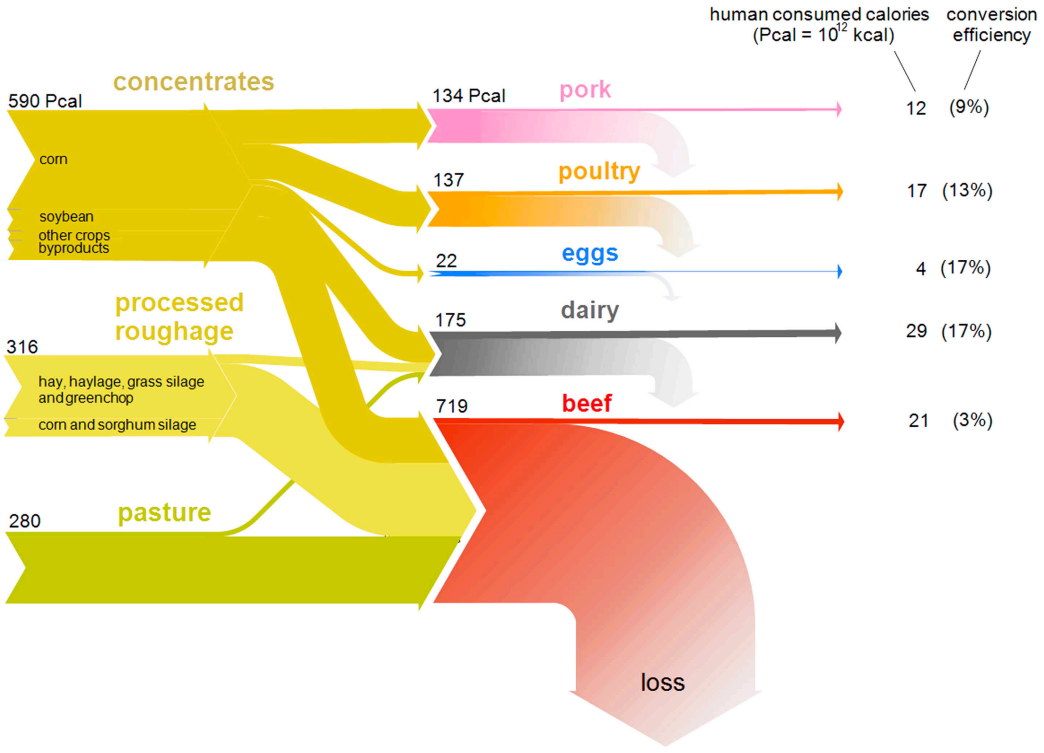

Figure 1. *Protein conversion of different food products (Shepon, Eshel et al. 2016).*

1.2 Rapeseed

Rapeseed is the second largest cultivated oilseed in the world and in 2013, 72.67 million tons of rapeseed was produced (Wanasundara, Tan et al. 2017). In Sweden, rapeseed is the most commonly grown oil seed crop and 3 254 400 tons of rapeseed was produced in 2018 (Jordbruksverket 2018). Rapeseed is primarily used for oil production (Wanasundara, Tan et al. 2017). The oil is released by mechanical separation of oil and solids. The residue from the oil production is called rapeseed press cake (RSPC) and consist mostly of proteins (30 %) and fibers. These proteins can be extracted and are interesting as a novel source of food due to their nutritional quality (Wanasundara, Tan et al. 2017). There is only one current food application with rapeseed protein on the European market, a rapeseed

isolate with 90 % proteins, and it was approved in 2013 by the European Food Safety Authority (EFSA 2013). There are two major production processes for rapeseed oil: hot-pressed or cold-pressed.

1.2.1 Hot-Pressed Rapeseed Meal

The first step in producing hot-pressed rapeseed oil is to clean the seeds from unwanted substances to keep a high quality of the oil. The seeds are then pre-heated to 35˚C to prevent the seeds from shattering during the following step. The seeds are flaked by a roller mill to rupture the seed coat. Thereafter, the flakes go through a cooking step (80-105°C) and are then pressed by a series of screw presses to extract the oil. Pressing can usually not extract all the oil, 16-20% is still left in the seeds, and thus solvents are usually used to recover the remaining oil (CCC 2016).

1.2.2 Cold-Pressed Rapeseed Press Cake

Firstly the seeds are cleaned from unwanted substances and dried to keep a high quality of the oil. The seeds are thereafter pressed by a series of screw presses and the temperature of the oil is never exceeding 35˚C. The press cake on the contrary has a temperature around 55-60°C when exiting the nozzle of the press. After pressing, fine solids are removed by a filter press. No solvents are used during production and the residual oil after the screw press remain in the press cake (Gunnarshög 2019).

1.2.3 Protein Quality in the Press Cake

Due to high concentrations of anti-nutrients (glucosinolates, phytates and phenolics) in the press cake, it is not suitable for human food production without further purification (Tan, Mailer et al. 2011). Therefore, the press cake is currently used for animal feed, even though the press cake contains a well-balanced amino acid composition and has a protein content of 30 % (Yoshie-Stark, Wada et al. 2008). The rapeseed protein also has a nutritional value comparable with soy (Wanasundara, Tan et al. 2017). Due to the nutritional quality and low climate footprint, this protein should be further investigated to be able to produce a new plant based product.

1.3 Project Background

Previous studies and master students have developed a method for protein recovery from cold-pressed rapeseed press cake. Very briefly, the rapeseed press cake is ground and soaked in a basic solution. In this step the proteins are being charged and are therefore solubilized in the aqueous phase. Thereafter follows a separation step where solids and aqueous solution are separated into two streams: a *heavy phase* containing husk, intact cells and insoluble proteins and a *light phase* with solubilized proteins and some fibers. After that the pH is adjusted to precipitate the proteins, the experiment follows with a step where the precipitated proteins are separated. Different basic pH have been investigated to achieve a high protein recovery. For the leaching step, pH 12 is known to yield the highest protein recovery. But for the process, pH [redacted] is used instead due to a lower environmental impact by using lower amount of NaOH and to cause less corrosion on the stainless equipment used in the process.

A variation of pH has also been investigated to evaluate how the precipitation pH affects the protein recovery. This master thesis started out with an initial screening study and found that at pH [redacted], the protein yield was slightly higher compared to pH [redacted] and [redacted]. Therefore, this present study used pH [redacted] for the precipitation step. It is not fully known how the protein is distributed in the different streams in the recovery process, and such information would be valuable information for the overall research to further increase the protein yield.

In a previous unpublished study performed by Östbring et al, the protein recovery from cold-pressed RSPC, hot-pressed rapeseed meal and hot-pressed rapeseed meal with hexane were compared. The cold-pressed RSPC yielded a significantly higher protein recovery than the other two. The lower

recovery for the hot-pressed rapeseed meal might be due to partial protein denaturation because of the high temperatures during oil production. The study suggested that cold-pressed rapeseed press cake is a better starting material for protein recovery compared with hot-pressed rapeseed meal. Therefore, only cold-pressed rapeseed press cake was studied in this master thesis.

1.4 Recirculation

The experiments in this thesis was performed in semi-pilot scale. The process was inspired by other food processes where recirculation of raw material is used to increase the yield of the process. For example, when anhydrous milk fat is produced, the cream is recirculated within the process to be able to ensure a high separation (TetraPak 2015). Moreover, recirculation has also been investigated when trying to improve extraction of oleosomes from soybean flour. A continuous centrifugation with a three-phased decanter was used in the process. The ingoing slurry that was not separated well enough was recirculated to the decanter until most of the oleosomes was successfully recovered from the soybean flour (Kapchie, Hauck et al. 2011). Inspired by this, a recirculation step was added to the current method to investigate if it could additionally increase the protein recovery.

1.5 Decanter

The first separation step in the process is executed by a continuous centrifuge, a two-phased decanter (Figure 2). A decanter can be used in different kinds of process industries such as food, chemical and pharmaceutical to produce high quality products (Lemitec 2019).

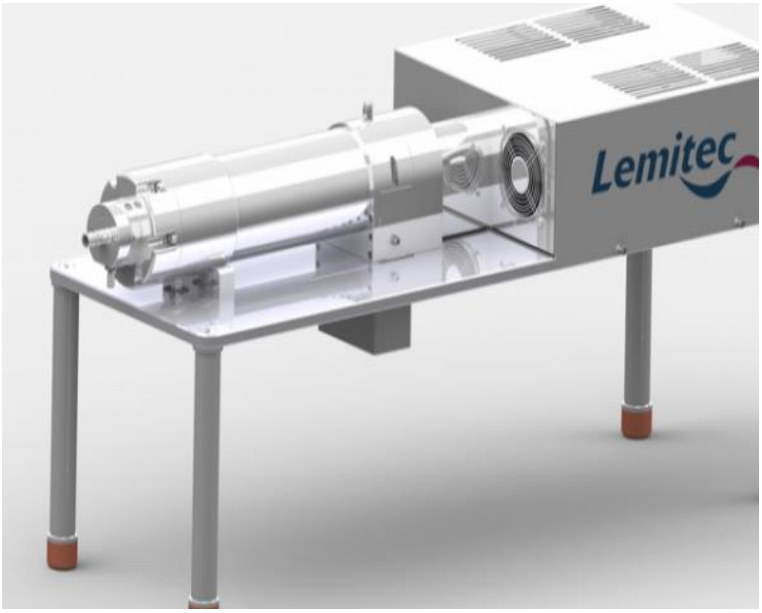

Figure 2. *A two-phased decanter from Lemitec (Lemitec 2019).*

The operating principle of a decanter is that the incoming slurry is pumped into the decanter and then accelerated. Centrifugal forces separates the slurry into a liquid phase (*light phase*) and a solid phase (*heavy phase*). The scroll inside the bowl rotates slightly faster than the bowl, causing the solids to convey towards the conical end (Figure 3). The separation factor depends on different parameters: flow of the feed, rate of revolution, differential forces, and the size of the weir disc that is used (Lemitec 2019).

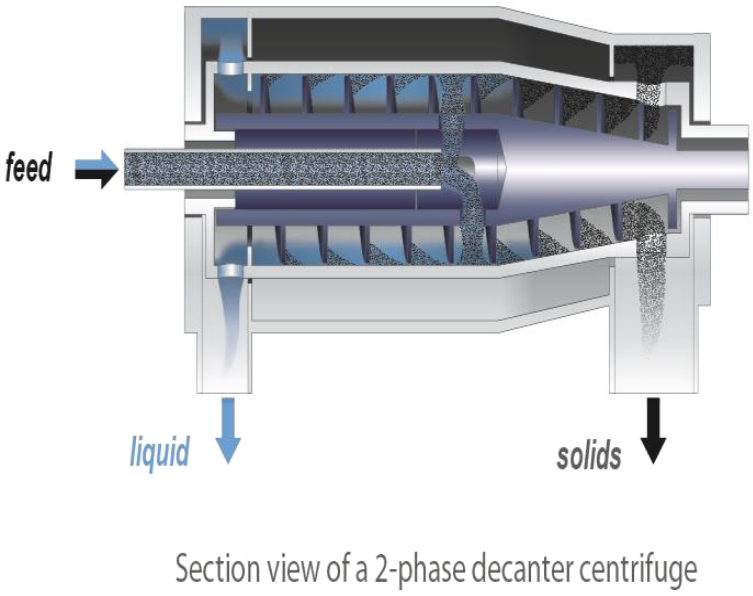

Figure 3. *Section view of a two-phased decanter.*

1.6 Aim

The aim of this master thesis can be divided into two parts.

1. Create a mass balance of the protein in the semi-pilot scale to map out its distribution in the different process streams and identify the losses during the process.
2. Investigate if the protein yield can be increased by serial recirculating of the heavy phase from the decanter.

1.7 Hypothesis

Since a reaction never will proceed to 100% and the reactants always will exist in an equilibrium, all protein in the rapeseed press cake will not be solubilized during the leaching step and therefore stay in the heavy phase. Recirculation of the heavy phase will therefore increase the protein yield.

2. Materials and Methods

2.1 Material and Chemicals

Cold-pressed rapeseed press cake (RSPC), a kind gift from Gunnarshögs Jordbruks AB, was used in all experiments. The screw press in the Gunnarshög plant yields cold-pressed oil that is never exceeding 37°C and the press cake has a temperature around 55-60°C when exiting the nozzle. The press cake was stored in -18°C before onset of the experiments.

Citric acid was purchased from Merck (Darmstadt, Germany) and aspartic acid was purchased from Thermo Electron (Milan, Italy).

2.2 Extraction of Protein from Rapeseed Press Cake

The first process step was leaching of protein. Rapeseed press cake (2000 g) was ground in a knife mill (Retsch Grindomix GM 200, Haan, Germany) for 3 minutes to a powder. The grinding was performed in portions of 500 g at the time. The powder was dispersed in tap water (1:10 w/w) in a stirred tank and the pH was adjusted to [redacted] by 2 M NaOH. After 10 minutes, the pH was re-adjusted to [redacted]. The slurry was stirred at 200 rpm for [redacted] hours (IKA RW 28 digital). The pH was noted after [redacted] hours to determine the pH drop during leaching. Typically, the pH dropped to [redacted]-[redacted] within the [redacted] hours incubation.

After leaching, a separation step was performed to separate husk and intact cells from solubilized proteins. The separation was carried out by a decanter (Decanter centrifuge DM80, Lemitec, Germany) with 6687 rates of revolution (2000·g) and differential force of 10 rpm. The weir disc was 52 mm. The flow to the decanter was adjusted to approximately 25 L/h with a peristaltic pump (Masterflex Easy-load Model 77200-62, Cole-Parmer, USA). The ingoing slurry was separated into two streams: a light phase and a heavy phase. The heavy phase was saved for recirculation (see section 2.2.1 below) and the light phase was collected for the next step in the experiment.

From the light phase, four test samples of approximately 400 g each were collected. The protein was precipitated by pH-adjustment to [redacted] with citric acid. The slurry was stirred with a magnetic stirrer for 20 minutes. Thereafter, the slurry was centrifuged for 20 minutes at 20°C at 5000·g. The sediment was collected as final product and the supernatant was discarded.

Samples for further analysis were collected throughout the process to the able to track the protein content. Samples were collected from the ground RSPC powder, the heavy phase, the light phase, sediment and supernatant from all four containers. All samples were kept in the freezer at -18°C until analysis.

2.2.1 Recirculation of Heavy Phase

To be able to extract even more proteins from the RSPC, the heavy phase from the decanter was recirculated. Instead of using the ground RSPC powder as starting material, the heavy phase from the previous day was used. The heavy phase was re-dispersed in tap water (1:10 w/w), the pH was adjusted to [redacted] and the experiment was repeated as described above. The dilution was made on wet weight, hence the absolute dilution factor was differing between the days. The experiment went on for 4 days, i.e. recirculation of the heavy phase three times. Samples for further analysis were taken every day from the heavy phase, the light phase, sediment and supernatant. The experiment in pilot scale was repeated for a total of 3 times, see Figure 4 for a schematic lineup and Figure 5 for an overview of the process.

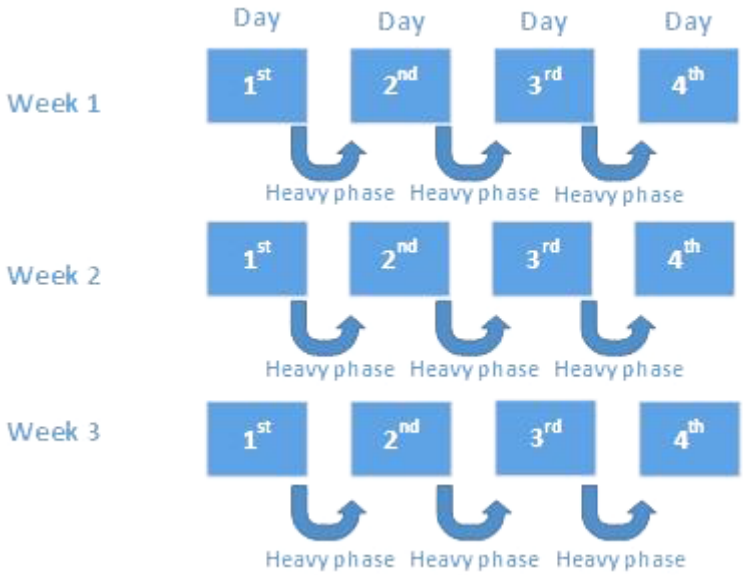

Figure 4. *Schematic lineup of the experiment.*

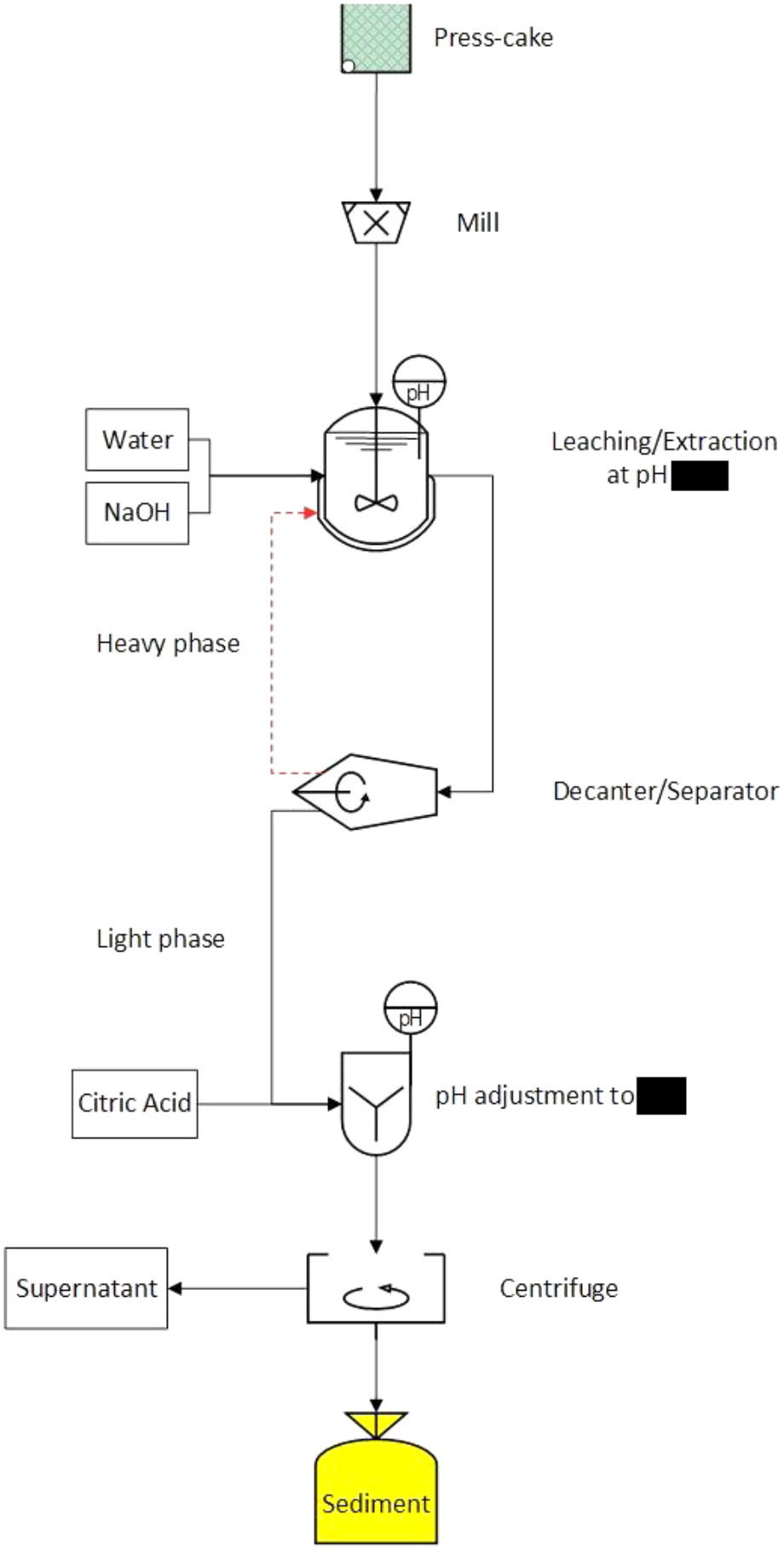

Figure 5. *A flowchart of the process used in the experiments. The red dashed arrow shows the recirculation of the heavy phase.*

2.3 Proximate Analysis

2.3.1 Dry Matter Content

Dry matter content was determined for all samples collected during the experiment. Separate methods were used for solid/semi-solid samples (RSPC, heavy phase and sediment) and liquid samples (light phase and supernatant).

Oven drying

To determine the dry matter content in the solid phases, the samples were dried in an oven. Metal cups were coated with aluminum foil and approximately four g of sample was placed in each cup. Thereafter, the cups were placed in an oven (Termaks, Bergen, Norway) at 103°C until reaching constant weight (approximately 24 hours). The samples were thereafter allowed to cool down to room temperature in a desiccator. The analysis was performed in duplicates.

The dry matter content on wet basis was calculated with equation 1.

$$Dry\ matter\ (\%) = \left(1 - \frac{Sample\ before\ drying\ (g) - Sample\ after\ drying\ (g)}{Sample\ before\ drying\ (g)}\right) \cdot 100\ \% \qquad (1)$$

Freeze drying

For the liquid samples, freeze-drying was used to determinate the dry matter content due to the very low amount of solids in these samples. Approximately 80 g of sample was put in each aluminum foil container. The samples were thereafter placed in the freeze dryer (Labconco Lyph Lock 18) for four days. To ensure that all water had evaporated, the samples were placed in the oven at 103°C for a minimum of one day after completed freeze-drying. The samples were then allowed to cool down to room temperature in a desiccator. The dry matter content on wet basis was calculated with equation 1.

2.3.2 Protein Quantification

The protein content was quantified for each sample by a protein analyzer (Thermo Electron Corp., Flash EA, 1112 Series). Air was used as blank and aspartic acid was used as reference. To be able to calculate the protein content from nitrogen, a conversion factor of 6.25 was used. Approximately 25-50 mg of sample was placed in a 33 mm tin disc and were thereafter placed in the protein analyzer. The analysis was performed in duplicates.

The protein yield was calculated in two ways, absolute and relative yield. Absolute yield correlates to the starting material RSPC and relative yield to the start material of the day the experiment was performed (heavy phase from the day before) (Figure 6). In this way, the absolute yield reflects how large proportion of the protein content in the RPSC that was recovered and the relative yield reflects the protein recovery efficacy for each recirculation.

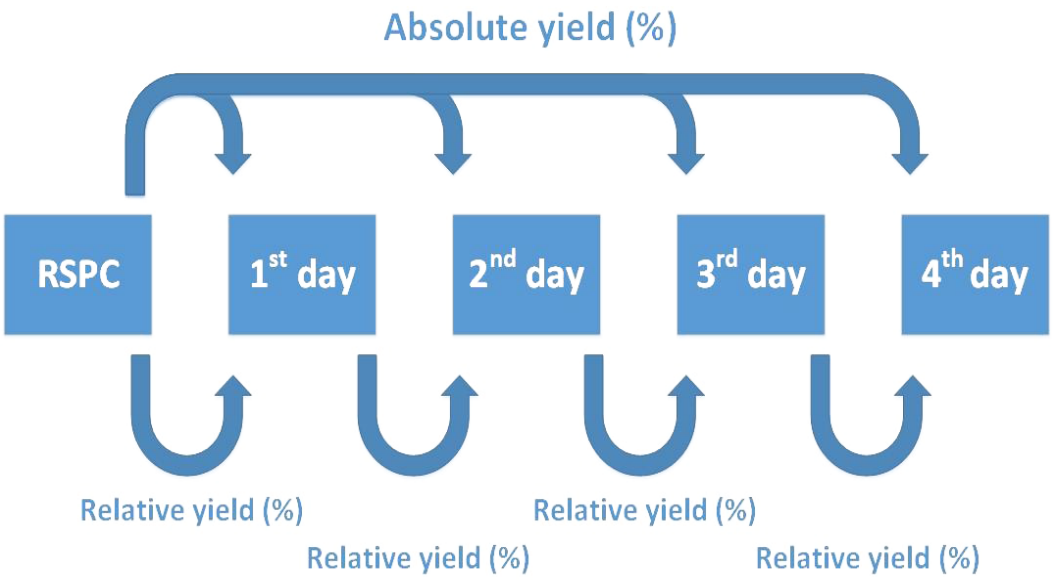

Figure 6. *Graphical illustration of absolute and relative yield.*

The absolute yield was calculated with Equation 2.

$$Absolute\ yield\ (\%) = \frac{Protein\ in\ sample\ (g)}{Protein\ in\ RSPC\ (g)} \cdot 100\ \% \quad (2)$$

The relative yield was calculated with Equation 3.

$$Relative\ yield\ (\%) = \frac{Protein\ in\ sample\ (g)}{Protein\ in\ heavy\ phase\ \ as\ start\ material\ (g)} \cdot 100\ \% \quad (3)$$

2.4 Statistical Analysis

All analysis were carried out in at least duplicates. Possible differences in means of absolute and relative protein yield were analysed using student *t*-test in excel. Results were considered significant if P values were < 0.05. All results are expressed as means with standard deviation.

3. Results and Discussion

3.1 Material Balances

Material balances for each day (Figure 7 and Appendix) were compiled to get an overview of the process and to get a numerical overview the protein distribution, total weight of each stream and the concentration of dry solids (Table 1).

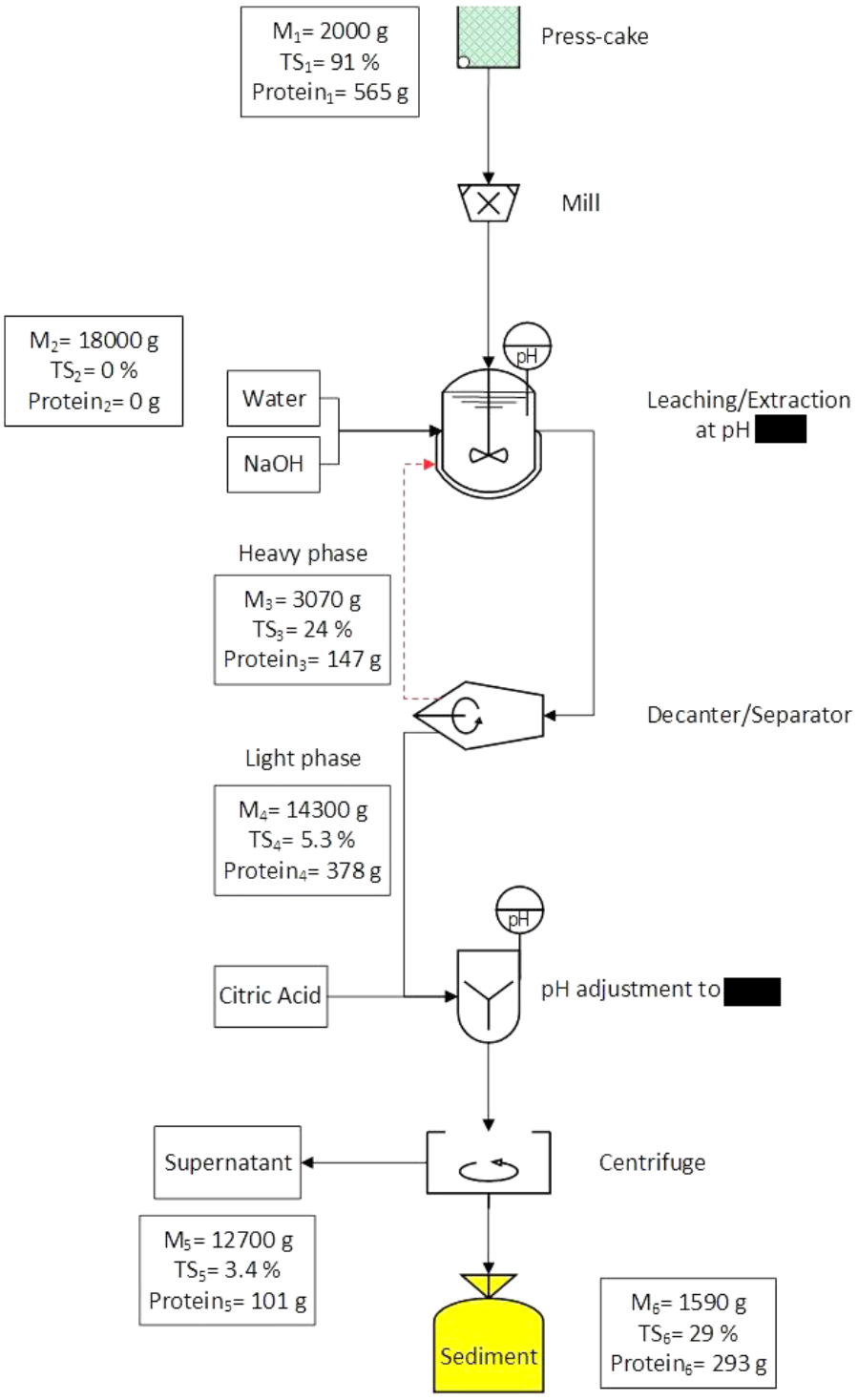

Figure 7. *Material balance of protein of the first day of the experiment. Total mass (g), dry matter content (%) and amount of protein (g) in the relevant streams are indicated in the Figure. Data are reported as mean of three runs on pilot scale.*

Table 1. *Total mass (g), dry solids (%), protein (g) in each stream for each day of the experiment.*

Stream	Variable	1st day	2nd day	3rd day	4th day
Start material	M_1 (g)	2000 ± 0.0	3070 ± 40	2140 ± 130	1330 ± 180
	TS_1 (%)	91 ± 0.6	24 ± 1	19 ± 0.1	19 ± 0.8
	P_1 (g)	565 ± 9.9	147 ± 10	56.4 ± 11	30.5 ± 5.5
Water	M_2 (g)	18000 ± 0.0	27300 ± 220	18900 ± 1200	12000 ± 1600
	TS_2 (%)	na	na	na	na
	P_2 (g)	na	na	na	na
Heavy phase	M_3 (g)	3070 ± 40	2140 ± 130	1330 ± 180	637 ± 220
	TS_3 (%)	24 ± 0.9	19 ± 0.1	19 ± 0.8	17 ± 0.02
	P_3 (g)	147 ± 10	56.4 ± 11	30.5 ± 5.5	13.9 ± 5.2
Light phase	M_4 (g)	14300 ± 1000	25100 ± 700	16300 ± 940	9010 ± 1500
	TS_4 (%)	5.3 ± 0.08	0.60 ± 0.04	0.11 ± 0.01	0.051 ± 0.03
	P_4 (g)	378 ± 20	53.9 ± 4.0	3.75 ± 0.21	na
Supernatant	M_5 (g)	12700 ± 930	24500 ± 660	16100 ± 930	8900 ± 1500
	TS_5 (%)	3.4 ± 0.2	0.37 ± 0.02	0.11 ± 0.02	0.075 ± 0.03
	P_5 (g)	101 ± 5.4	8.56 ± 1.8	0.614 ± 0.029	0.0982 ± 0.0015
Sediment	M_6 (g)	1590 ± 130	633 ± 34	246 ± 15	104 ± 40
	TS_6 (%)	29 ± 2	18 ± 1	8.1 ± 0.2	5.7 ± 2
	P_6 (g)	293 ± 24	52.0 ± 0.88	3.51 ± 2.0	1.01 ± 0.14

Each week in the experiment started with the same amount of rapeseed press cake (RSPC), 2000 g. The starting amount of protein in the RSPC was 565 g. After leaching and the first separation, the protein was divided between the heavy phase (husk, intact cells and non-solubilized proteins) and the light phase (solubilized proteins, some fat and fibers). 147 g of protein from the RSPC was found in the heavy phase and 378 g in the light phase, meaning that 67 % of all the proteins in the RSPC were successfully extracted. After the pH adjustment to [redacted] and the second separation, 293 g of the initial protein was found in the sediment (end product) and 101 g of protein was lost in the supernatant. Further presentation and discussion of the recirculation experiment follows further on.

3.2 Protein Yield

3.2.1 Absolute Protein Yield

The absolute yield of the first day of the experiment was 52 % (Figure 8), which means that 52 % of the protein in the RSPC was successfully extracted and could be recovered in the final sediment. Once the heavy phase from the first day was recirculated, an additional 9.3 % of the protein from the RSPC could be recovered. Further recirculation of the heavy phase only achieved an absolute yield of 1.0 % and 0.18 %, a relatively low yield in comparison with the first two days.

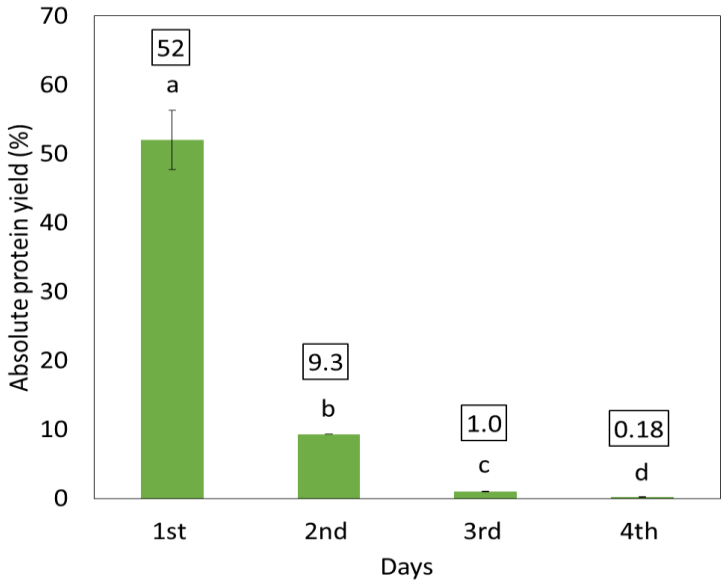

Figure 8. *The absolute protein yield (%) for each day. Different letters indicates significant differences.*

In a study that was performed by Manamperi et al in 2011, the effect on the protein recovery at different extraction and precipitation pH were investigated and simulated. According to their results, the yield obtained in this master thesis should not exceed 22 %. In the study by Manamperi et al, the leaching step only lasted for one hour compared to [redacted] hours in this present study. Moreover, they used hot-pressed rapeseed meal (Manamperi, Wiesenborn et al. 2011) that had been considerably warmer than the cold-pressed rapeseed press cake used in this study. Contrary to their result, by having a longer leaching step and using cold-pressed rapeseed press cake, a high protein recovery could still be obtained.

3.2.2 Relative Protein Yield

The relative yield for the first day is the same as for absolute yield, due to the fact that the yields are based on the same staring material, the RSPC. The relative yield for the second day was 35 % (Figure 9), which means that 35 % of the starting material (heavy phase from the previous day) could be recovered in the subsequent sediment. For the third and fourth day, the relative yield was only 11 % and 3.8 %; relatively low in comparison with the first and second day. Relative yield can be used as a measure of the efficiency of the process for each day. The first day, the protein extraction was relatively effective but for the second day, the efficacy was reduced. For the third and fourth day, a dramatic reduction of protein extraction efficacy could be seen.

It seems like it became more difficult to solubilize and precipitate the proteins for each additional recirculation. This can relate to the fact that the RSPC consists of a variety of different proteins that do not behave in the same way. All proteins may not be solubilized during the leaching step and therefore never leave the heavy phase. Others proteins might have an isoelectric point differing from pH [redacted]. These proteins do not precipitate at the chosen pH and therefore remain in the supernatant that gets discarded, resulting in a lower yield.

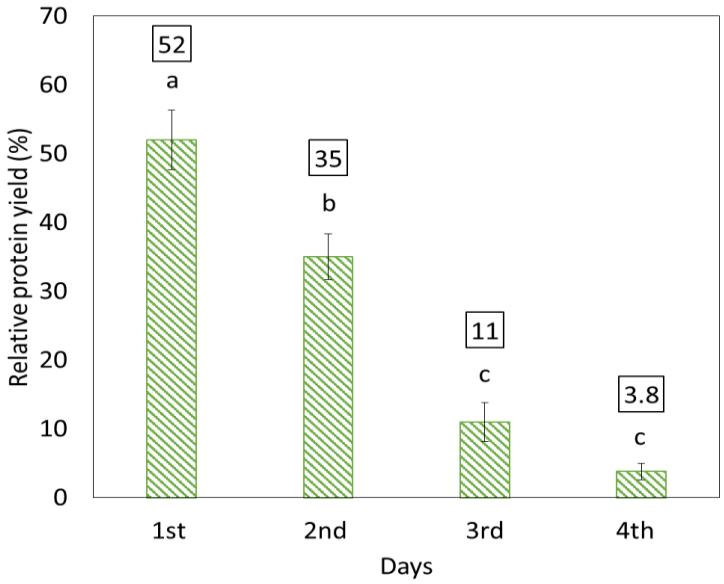

Figure 9. *The relative yield (%) of the sediment for each day. Different letters indicates significant differences.*

3.3 Protein Distribution in the Different Process Streams

The overall aim of the master thesis was to find strategies to increase the protein yield. In order to do so, an attempt was made to map the protein distribution in the different process streams (Figure 10 and Table 2). Samples were collected along the process and the protein content were quantified.

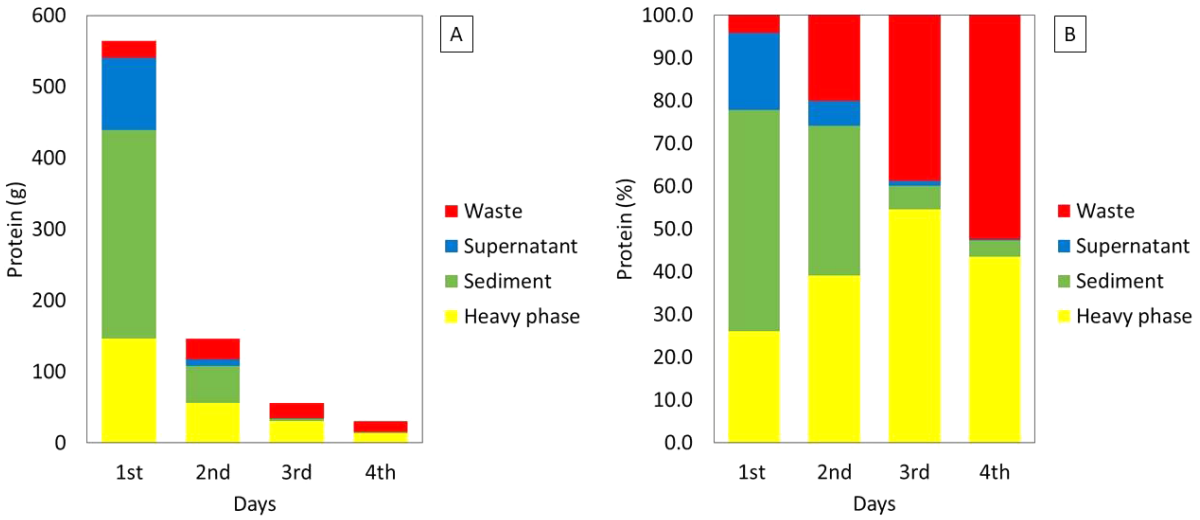

Figure 10. *An overview over protein distribution in the different streams for every day in the experiment.*

Table 2. *Protein distribution in each stream. Mass (g) is connected to Figure 10 A and percent to Figure 10 B.*

Days	Unit	1st day	2nd day	3rd day	4th day
Heavy phase	g	147 ± 10	56.4 ± 11	30.5 ± 5.5	13.9 ± 5.2
	%	26 ± 2	39 ± 10	55 ± 6	44 ± 10
Sediment	g	293 ± 24	52.0 ± 0.88	3.51 ± 2.0	1.01 ± 0.14
	%	52 ± 4	35 ± 3	5.6 ± 2	3.7 ± 1
Supernatant	g	101 ± 5.4	8.56 ± 1.8	0.614 ± 0.029	0.0982 ± 0.0015
	%	18 ± 1	5.8 ± 1	1.1 ± 0.1	0.35 ± 0.07
Waste	g	24.3 ± na	29.6 ± na	21.8 ± na	15.5 ± na
	%	4.0 ± na	20 ± na	38 ± na	52 ± na

3.3.1 Protein Distribution without Recirculation

The first day, after decantering, 378 g of the protein from start (565 g) was successfully extracted to the light phase (sediment and supernatant) (Table 1). That means that 67 % of the protein from the RSPC was extracted during the leaching step. The protein that was never extracted ended up in the heavy phase, 147 g (26 %) to be more exact. A chemical or biochemical reaction will never result in a 100 % yield due to losses during different steps of the process. As previously discussed (3.2.2 Relative Protein Yield), the RSPC consist of a variety of proteins that behave differently during the leaching step. Some proteins, at least the proteins on the surface of the RSPC pellet have been exposed for temperatures around 60°C and may have been subjected to denaturation. Denatured proteins cannot be fully extracted during the leaching step due to their altered biochemical structure and will therefore probably stay in the heavy phase during all the recirculation as well. Furthermore, previous studies (source: undergraduate BLTF01-reports, 2018) show that if the leaching step proceeded for 16 hours

instead of [redacted], more protein could be extracted and the yield would increase. Unfortunately, the end product had a dark color indicating that a reaction between the protein and the polyphenol may have occurred. Due to a more time consuming process and a darker color, a [redacted] hours leaching step was preferred.

A research group from Germany also investigated the extraction possibilities of cold-pressed rapeseed press cake. They investigated the effect of pH during extraction and also the time span of the leaching step. They used both hot-pressed rapeseed meal and cold-pressed RSPC in their experiments and compared the results. Their process was similar to the process in the present study. They received the highest yield (59.5 %) at pH 12 and the longer the leaching step proceeded, the higher protein yield was obtained (Fetzer, Herfellner et al. 2018). The research group focused on soluble proteins for technical applications such as biodegradable glues and plastic films, and their results can therefore be compared to the amount of proteins in the light phase in the present study. Fetzer et al. investigated only one parameter at the time; pH or time of the leaching step. Due to both [redacted] hours leaching step and pH [redacted], the present study could obtain 67% proteins in the light phase, fairly higher than their results.

After pH precipitation and centrifugation the first day, 293 g (52 %) of the protein was located in the sediment. Approximately one fourth, 101 g of protein, was never precipitated at pH [redacted] and was instead located in the supernatant after centrifugation. This results in a waste of 18 % of the protein being discarded. To further increase the overall yield, other strategies should be used to recover these proteins as well. The loss can be due to the variety of proteins in the RSPC. The proteins with an isoelectric point at [redacted] will precipitate at pH [redacted], but the other proteins will remain in the supernatant.

In a study performed by Ghodsvali et al in 2005, different precipitation pH values were investigated to find the isoelectric point that generated the highest protein recovery. The study used hot-pressed rapeseed meal instead of cold-pressed rapeseed press cake, and pH 12 for the leaching step instead of pH [redacted] as was used in the present study. In the study reported by Ghodsvali et al, the rapeseed meal was dispersed in water for 30 minutes, followed by basic incubation at pH 12 and centrifugation. They obtained the highest protein precipitation, around 60 %, at pH 4.5-5.5 in preference to [redacted]. They performed their experiments in lab scale and accounted both the sediment and supernatant as end products (Ghodsvali, Khodaparast et al. 2005). The present study was performed in semi-pilot scale, and could obtain a protein yield of 52 % with just the precipitated final sediment as the end product. If supernatant would be considered an end product as well, the protein yield for this particular study would be 67 %. One of the reasons that a relatively high yield could be obtained in the present study compared to Ghodsvali et al. could be due to the use of cold-pressed RSPC instead of hot-pressed rapeseed meal. The [redacted] hours leaching step probably contributes to the higher yield in the present study.

The precipitation can also be performed in a step-wise manner. Pedroche et al (2004) isolate protein from *Brassica carinata* (a close relative to rapeseed). The oil was extracted from the seeds together with a solvent, absent from heat treatment. Pedroche et al performed the precipitation in two steps, first at pH 5.0 and secondly at pH 3.5 to study the protein solubility (Pedroche, Yust et al. 2004). Unfortunately, the protein recovery yield was not reported so no further comparisons of the outcome can be made. By investigating the step-wise manner, the loss in the supernatant might be reduced and the protein yield could be increased. This should be investigated in the future.

In 1997, Klockeman et al performed a highly cited experiment with hot-pressed rapeseed meal, where over 99 % of the proteins from the press cake was extracted. They used both NaOH and $CaCl_2$ during the leaching step that proceeded for 60 minutes in baffled flask on an orbital shaker. They diluted the slurry until the concentration of NaOH was 0.4% but the exact pH was not reported. After precipitation

at pH 3.5, addition of NaCl and separation by a centrifuge, a protein recovery of 87.5% was obtained. The protein recovery includes both the sediment and supernatant. Worth mentioning is that they calculate their protein recovery from the soluble proteins from the rapeseed press cake, not the total protein content in the rapeseed press cake itself (Klockeman, Toledo et al. 1997). Therefore, their results are difficult to compare with the results from the present study. Although, the fact that they were able to extract over 99 % from the rapeseed press cake is impressing and might be due to addition of $CaCl_2$ during the leaching step, which increased the ion strength (Klockeman, Toledo et al. 1997). This step should be further investigated to see if addition of $CaCl_2$ could increase the extraction from cold-pressed rapeseed press cake as well. Moreover, precipitation with NaCl together with acid might increase the yield in this present study too.

3.3.2 Effect of Recirculation

On the second day, the experiment started with 3070 g heavy phase from the day before which contained 147 g of protein (Table 2). While 60.6 g protein was successfully extracted into the light phase, 56.4 g (39 %) protein was never extracted and were still in the heavy phase after decantering. In comparison with the first day where only 26 % ended up in the heavy phase, 39 % is quite high. Also, in contrast to the first day, almost all the protein in the light phase was precipitated at pH [redacted] and 52.0 g of protein was detected in the sediment. Only 8.56 g of protein was detected in the supernatant. The absolute yield for the first day was 52 % and 9.3 % for the second day. By recirculation of the heavy phase once, the absolute yield could be increased from 52 % to 61 %.

In the study performed by Pedroche et al (2004) mentioned above, they also investigated the effect of recirculation. They recirculated their material once to maximize the extraction from the heavy phase. At pH 10 after one recirculation, 55 % of the protein was extracted and at pH 11, 78 % of the protein was extracted (Pedroche, Yust et al. 2004). In this study, 378 g of protein was extracted from the press cake and subsequently located in the light phase after extraction the first day. It means that 67 % of the proteins from the rapeseed press cake, which contained 565 g of protein, was extracted during the leaching step. To be able to compare the results from Pedroche et al, the proteins from the first recirculation was included, that is an additional 54 g of protein. Thereby, the total amount of extracted protein was 431 g. It means that 76 % of the protein from the cold-pressed rapeseed press cake was extracted with one recirculation. That is almost as high as Pedroche et al got from leaching at pH 11 although the present study used the lower pH [redacted]. The reason for the relatively high proportion of extracted proteins in the present study can be due to the fact, that in contrast to Pedroche et al., no solvents were used. Solvents can denature some of the protein, which prevents these proteins to be solubilized during the leaching step. Another difference was that they used *Brassica carinata* and not rapeseed.

For both the third and fourth day, only a very limited amount of protein was extracted in the leaching step (green and blue in Figure 10) and most of the protein was still to be found in either the heavy phase (yellow) or was wasted in the process (red). This is illustrated in Figure 10 (B), where the amount of protein in the sediment and supernatant are significantly lower compared to the amount protein in the heavy phase and the waste part.

The waste in the process was almost in the same range for all four days in the experiment (red in Fig 10). The stirred tank was coned in the bottom and the pump outlet was located approximately 10 cm up from the bottom. This geometry always resulted in some slurry left in the tank that did not enter the decanter. Also, some material got stuck inside the decanter in every run. Both these fractions are counted as waste in the calculations. It seems that the combined waste is constant due to the equipment design and when the absolute amount protein in the system is decreasing, the proportion of waste is increasing (red in Figure 10).

3.4 Composition in Dry Matter

3.4.1 Sediment

The amount of solids in the sediment (end product) decreased for every recirculation (Figure 11 A and Table 3). The first day of the experiment, the protein content of the dry matter was 63 % (Figure 11 B) and had a total weight of 53.7 g. The second day, both the amount dry solids and protein content were drastically reduced compared to the first day. The protein content was only 44 % and the dry matter had a total weight of 7.62 g. The results from the third and fourth day were significantly lower in comparison with the first two days. Altogether, the relative amount of non-nitrogen substances increased over the four days (Figure 11 B and Table 3).

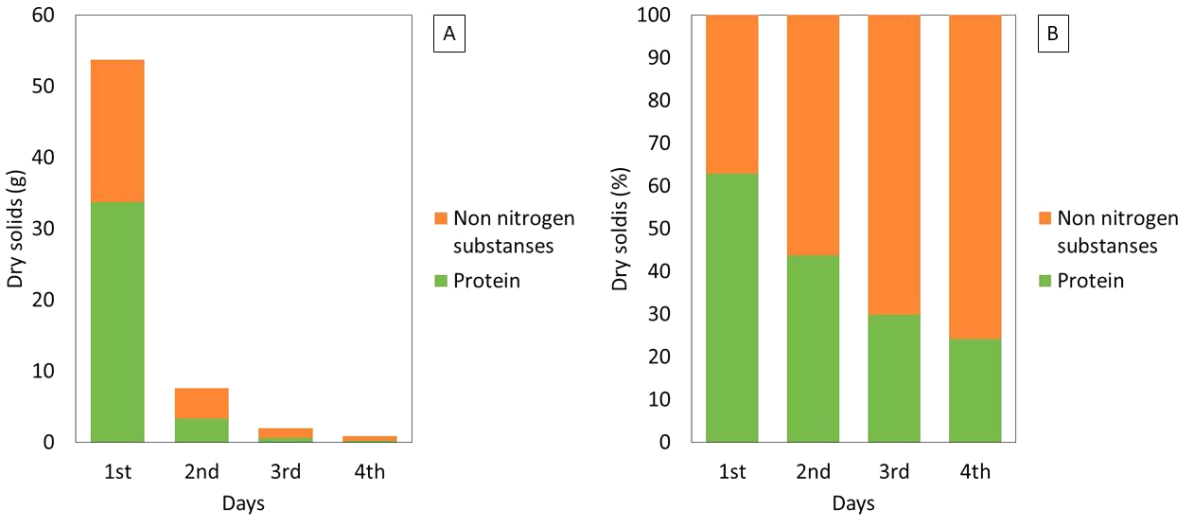

Figure 11. *The dry matter content of the sediment for each day of the experiment.*

Table 3. *The dry matter content of sediment.*

Days	Unit	1st	2nd	3rd	4th
Protein	g	33.7 ± 0.57	3.33 ± 0.13	0.589 ± 0.053	0.210 ±0.053
	%	63 ± 3	44 ± 0.1	30 ± 0.2	24 ± 0.2
Non nitrogen substances	g	20.0 ± 2.4	4.29 ± 0.18	1.39 ± 0.14	0.661 ± 0.16
	%	37 ± 3	56 ± 0.1	70 ± 0.2	76 ± 0.2

One of the reasons that the final protein concentration is of interest is due to further applications. To be able to texturize the protein, the protein content needs to be over 70 %. Otherwise the non-nitrogen substances, which consist mostly of starch and fibers, will disturb the formation of a texturized meat-like structure. Moreover, the only rapeseed protein product that has been approved by EFSA (European Food Safety Authority) was a rapeseed protein isolate with 90 % protein (EFSA 2013). Since this experiment resulted in a protein concentration of 63 %, further experiments need to be performed to be able to reach a higher content of protein in the final sediment. Another solution could be to add other plant proteins e.g. for example pea protein, oat protein or equivalent proteins, to increase the protein concentration in order to texturize the protein mix.

In a study performed by Yoshie-Stark (2008), two different methods were investigated for producing protein isolates. Both methods started their process similar to the present study but their extraction and precipitation pH differ. They used pH 7.4 at the leaching step and executed the precipitation at pH 5.8. Both methods used a pasteurization step before spray drying their end product and one method used an ultrafiltration step before pasteurization. The protein isolate from the method with the ultrafiltration step had a protein content of 98.7 % and the isolate without filtration had a protein content of 70.8 % (Yoshie-Stark, Wada et al. 2008). Even though they used different pH and their process differs, an ultrafiltration step should still be investigated for the present study as well. By using filtration, the non nitrogen substances in the isolates might be reduced and the end-product would get a higher protein content.

3.4.2 Supernatant

The amount of dry solids in the supernatant decreased for every recirculation (Figure 12 A and Table 4). For all four days, the non nitrogen substances in the supernatant were dominating and the relative protein content was reduced over time (Figure 12 B and Table 4). As the sediment was considered end product in the present study, protein was not wanted in the supernatant and the low protein content in this stream was a positive result. Although, some protein was still left in the supernatant. As previously discussed, this might be due to different isoelectric points for the rapeseed proteins. Starch and fibers will not precipitate at a certain pH and are therefore mostly found in the supernatant.

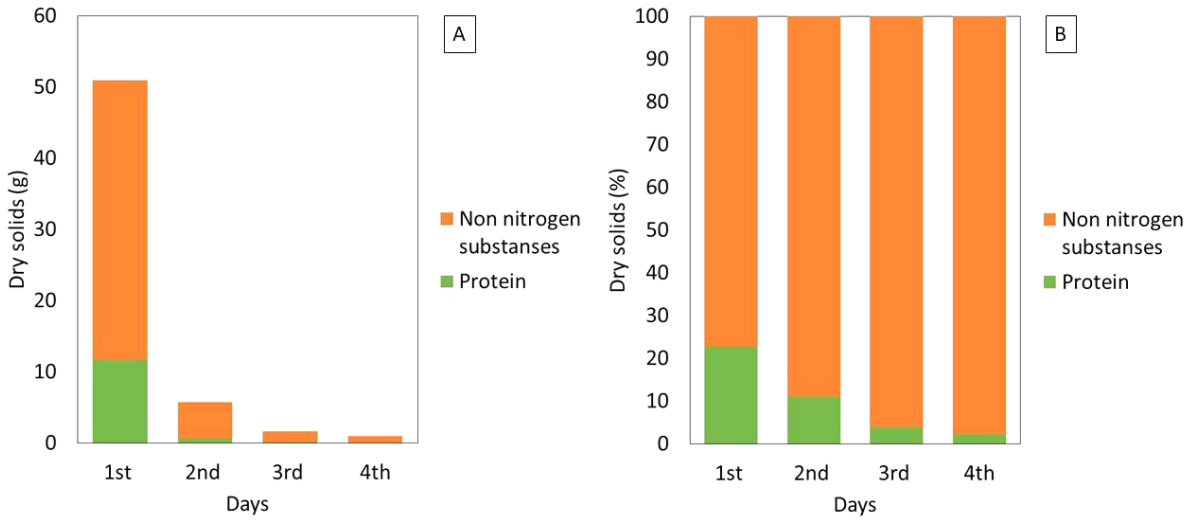

Figure 12. *The dry matter content of the supernatant for each day of the experiment.*

Table 4. *The dry matter content of supernatant.*

Days	Unit	1st	2nd	3rd	4th
Protein	g	11.6 ± 0.34	0.626 ± 0.029	0.0616 ± 0.00044	0.0202 ±0.0020
	%	23 ± 0.5	11 ± 0.5	4.0 ± 0.1	2.0 ± 0.1
Non nitrogen substances	g	39.4 ± 0.043	5.13 ± 0.0013	1.54 ± 0.046	0.944 ± 0.044
	%	77 ± 0.5	89 ± 0.5	96 ± 0.1	98 ± 0.1

The first day of the experiment, the protein content in the supernatant was 23 % and the dry matter had a total weight of 51.0 g (Figure 12 A). For the second day, both the protein content and the total weight was drastically decreased. The protein content was only 11 % and the total weight was 5.76 g. For both the third and fourth day, the dry matter mostly consisted of non nitrogen substances and the weight was almost non-existing.

Since 18 % of the proteins from the RSPC (Table 2) ended up in the supernatant the first day, these proteins should be recovered and not thrown away. The supernatant could be freeze-dried or spray dried and the protein could be formulated to a powder. In this stream, non-nitrogen compounds (probably most fibers) is dominant and can either be removed by filtration prior to drying, or left in the stream and then dried. The high concentration of fibers can contribute to health benefits in the human gut. Dietary fiber can be either soluble or insoluble. Soluble fibers are known to have a positive effect on the gut microflora and insoluble fibers can counteract constipation (Rayner, Östbring et al. 2016). To utilize also this fraction of the rapeseed, alternative applications such as drinks can be considered.

4. Conclusion

A mass balance over the process was created charactering the protein distribution in the various process streams. The majority of the proteins the first day (52 %) ended up in the sediment as desired. Protein losses in the process was protein that was never solubilized during the leaching step and therefore remained in the heavy phase (24 %) and proteins that would not precipitate at pH [redacted] thereby remained in the supernatant (18 %). Recirculation of proteins in the heavy phase resulted in an additional yield of 9.3 %. By recirculating the non-solubilized proteins in the heavy phase to a new leaching step, the yield could be raised from 52% to 61%. The majority of the remaining proteins after the first recirculation either ended up as waste or remained in the heavy phase throughout the experiments. The conclusion that can be drawn from the results is that recirculation of the heavy phase is definitely worth doing once to increase the protein recovery yield. A significant amount of protein was lost to the supernatant and the iso-electric points for these proteins should be further investigated to be able to precipitate them and possibly reach an even higher protein recovery yield.

5. Future Work

There are still plenty of different parameters within this master thesis that should be further investigated. The equipment of the process should be optimized to minimize the loss during processing. Since the stirred tank was coned in the bottom, some slurry was always left at the bottom. A different tank might lower the waste, or to change the pump outlet to a point closer to the bottom. The settings of the decanter were chosen to optimize separation of solubilized proteins from husk and intact cells. The settings should be looked over to be able to lower the waste inside the decanter but also to lower the amount of fibers in the light phase. Today, the slurry entering the decanter has a high flow (around 20 l/h) and the rpm are only 2000·g due to the noise caused by the decanter. The differential forces are only 10 rpm and the weir disc is 52 mm. By investigating the influence of the differential force and the size of the weir disc, the amount of fibers could possibly be reduced and thereby a higher protein concentration could be achieved in the final sediment. Also, if an ultra-filtration step would be included in the process, the amount of fibers needs to be reduced as well. Otherwise, the pores inside the ultra-filtration membrane could get clogged and thereby obstruct the concentration of proteins.

If the right decanter settings are found, the second separation step could also be performed with the decanter. During the master thesis work, a similar protein extraction experiment was performed with fava bean together with a Ph.D. student from Chalmers. The settings for the second centrifugation step after precipitation was set as follows: the flow of the slurry was lowered to 10 l/h, the rpm increased to 4000·g, the differential forces was set to 50 rpm and the same weir disc (52 mm) was used. This resulted in a successful separation and sediment as the end product could be collected. Therefore, similar settings should be used to perform the second separation step with the light phase after being precipitated with citric acid.

Since pH 12 is known to generate the highest protein recovery and this process uses [redacted] instead, a pH-control unit should be used during the leaching step to keep the pH constant. Today the pH usually drops to [redacted] during the [redacted] hours of leaching so if the pH could be hold constant at pH [redacted] instead, a higher protein recovery might be possible.

The precipitation step should also be investigated further in order to find the best precipitation pH or perhaps a combination of pHs. The precipitation could be executed in a step-wise manner to increase the protein recovery and thereby reduce the proteins being wasted to the supernatant. Other techniques could also be investigated, such as precipitation with salt.

6. Acknowledgment

I would first like to thank my supervisor, Dr. Karolina Östbring for all the encouragement during my work with my master thesis as well as for answering all of my questions during experiments and all the many valuable comments on my report. I would also like to thank my examiner Prof. Marilyn Rayner, researcher Ia Rosenlind and Ass Prof. Ingegerd Sjöholm for all your support and all of you for giving me the opportunity to join your rapeseed research team during the last six months. This has really been the highlight of my education!

I would like to thank Dan Johansson and Hans Bolinsson for answering all of my equipment related questions and for all the help you gave me. I would also like to thank the people at the food department and all the other master students for all the encouragement. Especially Ph.D. student Kajsa Nilsson who always took the time to give me a second opinion.

I would also like to thank Erik and Anja Nilsson at Gunnarshög for providing me with cold-pressed rapeseed press cake for all my trials.

Finally, I would like to thank all of my friends and family of unconditional support and for never doubting me. You are the reason I soon can call myself an engineer.

7. References

CCC (2016). "Steps in Oil and meal processing." Retrieved 16th jan, 2019, from https://www.canolacouncil.org/oil-and-meal/what-is-canola/how-canola-is-processed/steps-in-oil-and-meal-processing/.

EFSA (2013). "Scientific Opinion on the safety of “rapeseed protein isolate” as a Novel Food ingredient." EFSA Journal **11**(10).

Fetzer, A., et al. (2018). "Influence of process conditions during aqueous protein extraction upon yield from pre-pressed and cold-pressed rapeseed press cake." Industrial Crops and Products **112**: 236-246.

Ghodsvali, A., et al. (2005). "Preparation of canola protein materials using membrane technology and evaluation of meals functional properties." Food Research International **38**(2): 223-231.

Gunnarshög (2019). "Tillverkning." Retrieved 17th jan, 2019, from https://www.gunnarshog.se/tillverkning?infopath=9.

Jordbruksverket (2018). "Skörd av spannmål, trindsäd, oljeväxter och slåttervall 2018." Retrieved 16th jan, 2019, from https://www.jordbruksverket.se/webdav/files/SJV/Amnesomraden/Statistik,%20fakta/Vegetabilieproduktion/JO19/JO19SM1802/JO19SM1802_ikortadrag.htm.

Kapchie, V. N., et al. (2011). "Process Improvement for Semipurified Oleosomes on a Pilot-Plant Scale." Journal of Food Science **76**(6): C853-C860.

Klockeman, D. M., et al. (1997). "Isolation and Characterization of Defatted Canola Meal Protein." Journal of Agricultural and Food Chemistry **45**(10): 3867-3870.

Lemitec (2019). "Decanter centrifuges." Retrieved 18th jan, 2019, from http://www.lemitec.com/en/products/.

Lemitec (2019). "Lemitec MD 80 decanter centrifuge." Retrieved 18th jan, 2019, from http://www.lemitec.com/en/products/md80.php.

MacRae, R., et al. (2013). "The Food System and Climate Change: An Exploration of Emerging Strategies to Reduce GHG Emissions in Canada." Agroecology and Sustainable Food Systems **37**(8): 933-963.

Manamperi, W. A. R., et al. (2011). "Effects of Protein Separation Conditions on the Functional and Thermal Properties of Canola Protein Isolates." Journal of Food Science **76**(3): E266-E273.

Pedroche, J., et al. (2004). "Brassica carinata protein isolates: chemical composition, protein characterization and improvement of functional properties by protein hydrolysis." Food Chemistry **88**(3): 337-346.

Rayner, M., et al. (2016). Application of Natural Polymers in Food. Natural Polymers. O. Olatunji, Springer**:** 115-161.

Shepon, A., et al. (2016). "Energy and protein feed-to-food conversion efficiencies in the US and potential food security gains from dietary changes." Environmental Research Letters **11**(10).

Tan, S. H., et al. (2011). "Canola Proteins for Human Consumption: Extraction, Profile, and Functional Properties." Journal of Food Science **76**(1): R16-R28.

TetraPak (2015). Dairy Processing Handbook, Tetra Pak Processing Systems AB.

Wanasundara, J. P. D., et al. (2017). Proteins From Canola/Rapeseed: Current Status. Sustainable Protein Sources**:** 285-304.

Yoshie-Stark, Y., et al. (2008). "Chemical composition, functional properties, and bioactivities of rapeseed protein isolates." Food Chemistry **107**(1): 32-39.

Appendix

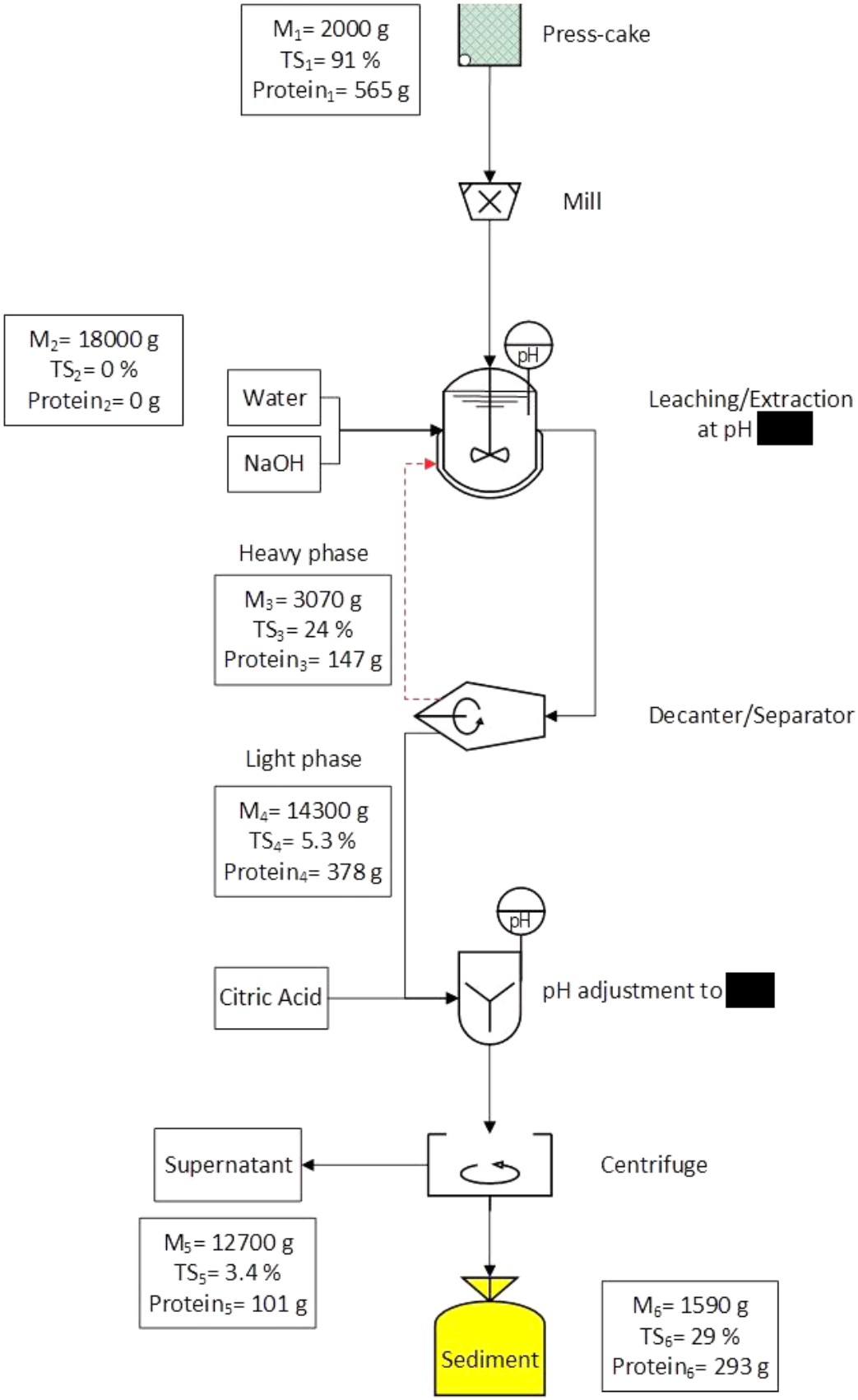

Figure 1. *Material balance of protein of the first day of the experiment. Total mass (g), dry matter content (%) and amount of protein (g) in the relevant streams are indicated in the Figure. Data are reported as mean of three runs on pilot scale.*

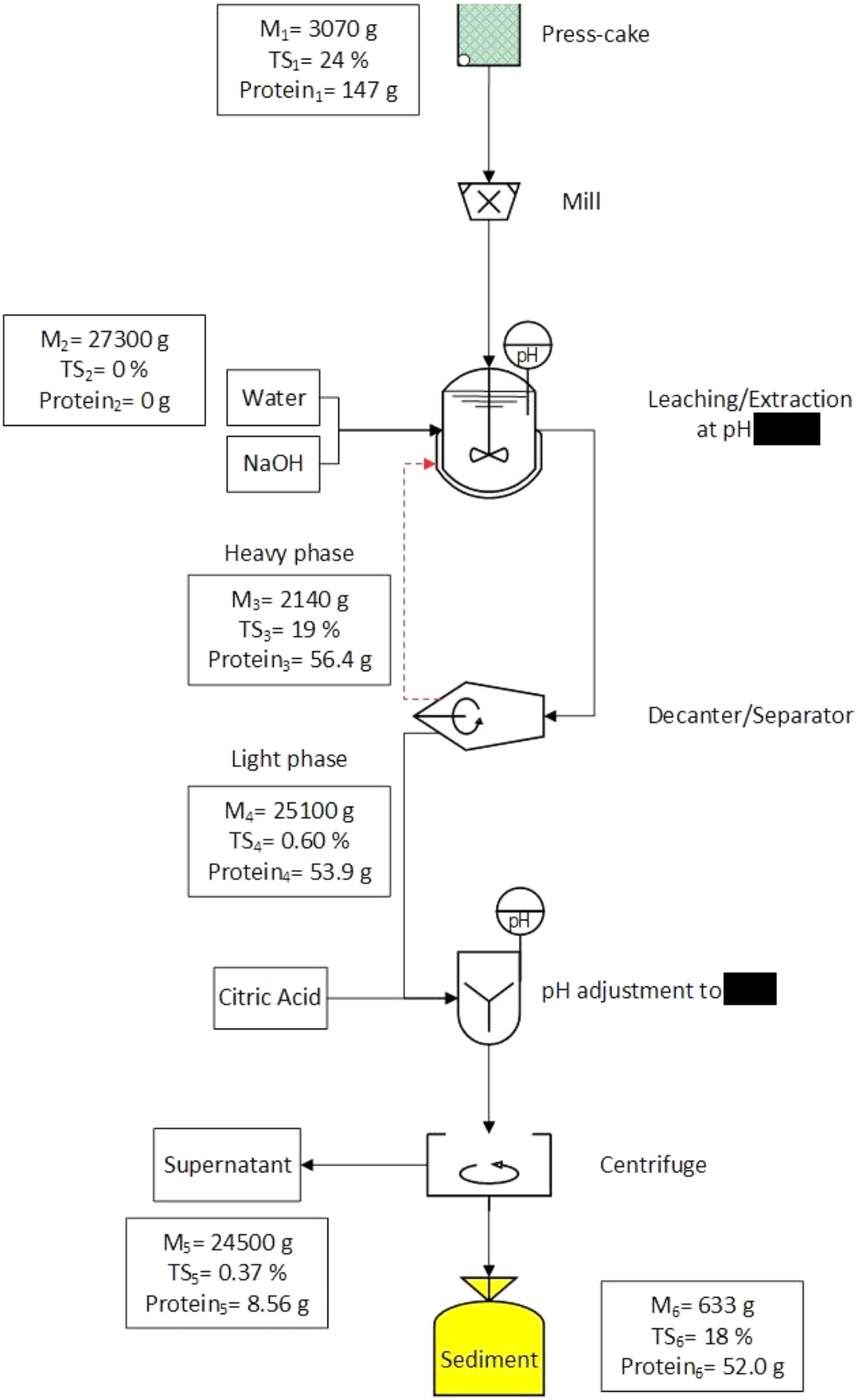

Figure 2. *Material balance of protein of the second day of the experiment. Total mass (g), dry matter content (%) and amount of protein (g) in the relevant streams are indicated in the Figure. Data are reported as mean of three runs on pilot scale.*

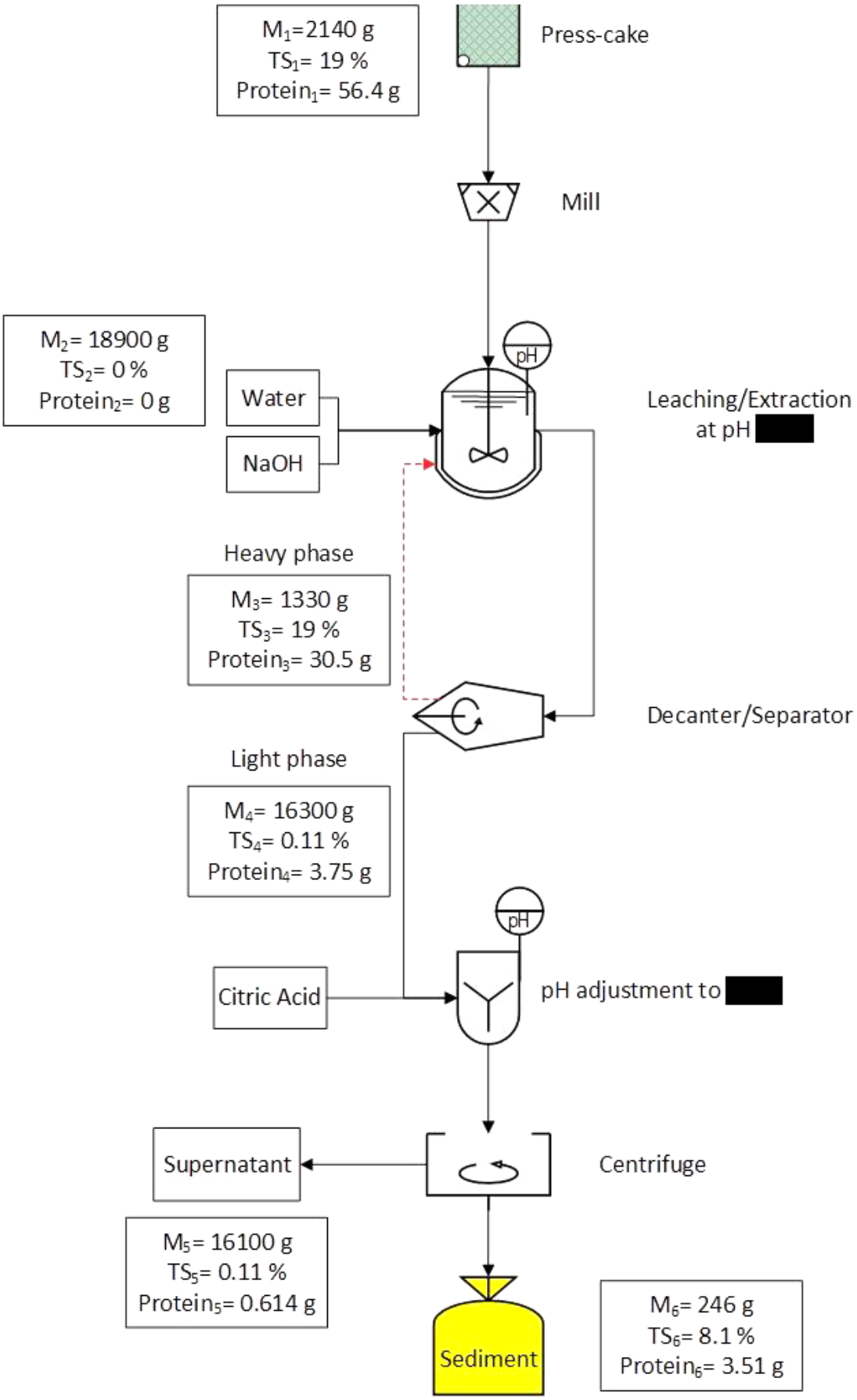

Figure 3. *Material balance of protein of the third day of the experiment. Total mass (g), dry matter content (%) and amount of protein (g) in the relevant streams are indicated in the Figure. Data are reported as mean of three runs on pilot scale.*

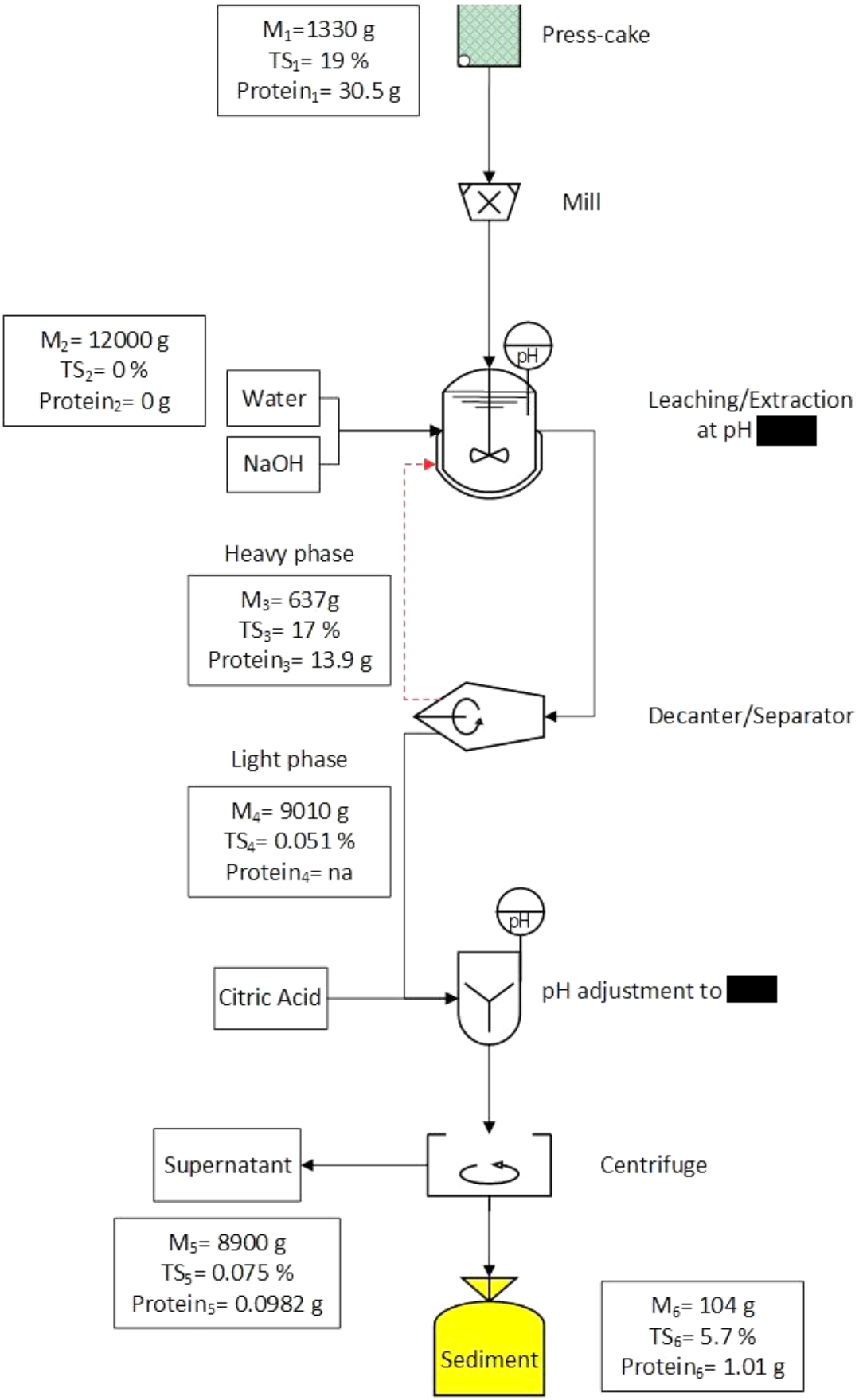

Figure 4. *Material balance of protein of the fourth day of the experiment. Total mass (g), dry matter content (%) and amount of protein (g) in the relevant streams are indicated in the Figure. Data are reported as mean of three runs on pilot scale.*