LUND UNIVERSITY

LTH

FACULTY OF ENGINEERING

Study of the effects of the operating conditions of a decanter centrifuge on a pilot-scale protein recovery setup from cold-pressed rapeseed press cake

DEPARTMENT OF FOOD TECHNOLOGY, ENGINEERING AND NUTRITION | LUND UNIVERSITY | SERGIO ALEJANDRO DE LA CRUZ PADILLA | MSc. IN FOOD TECHNOLOGY AND NUTRITION, SPRING 2023

LTH
FACULTY OF
ENGINEERING

Study of the effects of the operating conditions of a decanter centrifuge on a pilot-scale protein recovery setup from cold-pressed rapeseed press cake

Student:
Sergio Alejandro de la Cruz Padilla

Supervisor:
Cecilia Ahlström, PhD student (Main supervisor)
Ia Rosenlind (Co-supervisor)

Examiner:
Asst. Prof. Karolina Östbring

Acknowledgement

Firstly, I would like to express my gratitude to my family for being my unconditional support throughout my journey in Sweden. It is because of them that I found myself pursuing my master's degree at Lund University. I express my sincere gratitude to my supervisors, PhD student Cecilia Ahlström and Ia Rosenlind, for the unwavering support and guidance that they provided me throughout the planning and execution of my graduation project. Their invaluable input and feedback greatly enhanced the quality and significance of my research and aided my personal and academic growth. I am also deeply grateful to my examiner, Asst. Prof. Karolina Östbring, whose encouragement and expertise motivated me to explore the field of plant-based proteins and expand my knowledge. I would also like to thank my friends and fellow students who made my student journey a life-changing experience, especially my roommate and friend Kwanele Mkatini for his support during my day-to-day life in Lund. Special thanks also to the people from the Department of Food Technology, Engineering and Nutrition at Lund University who, in one way or another, helped me. Lastly, special mention to Gunnarshögs Jordbruks AB for providing me with the cold-pressed rapeseed press cake needed for the execution of this research.

Abstract

There is significant evidence to suggest that diets that include high levels of meat consumption have negative effects on both the environment and human health. As a result, efforts are being made to identify and characterize more plant-based proteins, such as those found in rapeseed. Rapeseed is the second-largest oilseed crop worldwide and is primarily used to produce edible oils through mechanical separation methods. During this process, a protein-rich by-product called press cake is produced, which is currently utilized as a source of protein in animal feed. Rapeseed press cake primarily consists of fibre (30%), protein (27-30%) and residual oil (20%). The use of rapeseed protein in food is limited because of its bitter taste and the antinutritional components it contains. In order to effectively recover protein from rapeseed press cake, it is necessary to scale up the process beyond laboratory-scale setups. Researchers from the Department of Food Technology, Engineering and Nutrition at Lund University have successfully achieved this; consequently, the current study aimed to further enhance the process's capacity and protein recovery yield in a pilot-scale setup.

The study aimed to recover proteins from rapeseed press cake using the salt extraction method at a concentration of 0.25M and a pH of 7, followed by isoelectric precipitation at pH 3.5 and a decanter centrifuge. The optimal processing parameters for the decanter centrifuge during the first protein recovery step were an inlet feed rate of 60 L/h, 2000xg acceleration, 50 rpm differential speed, and a weir disc with an inner diameter of 68 mm. A pH of 7 was suitable for a gentler extraction of proteins, with the addition of sodium chloride resulting in an extraction coefficient of 61%. Based on thorough analysis, it was determined that the most suitable set of processing parameters for the second phase of protein recovery were an inlet flow rate of 10 L/h, 4000xg acceleration, 6 rpm differential speed, and a 56 weir disc inner diameter. The latter enhanced the overall process capability without any adverse effects on critical factors such as dry matter and protein content of the precipitate. The study found that scraping equipment surfaces resulted in a significant increase in protein recovery yield. The yield increased from 17% to 29% under the selected processing conditions.

Popular science summary

Studies show that eating a lot of meat can be bad for both the environment and human health. The world is getting warmer, and it's important to find ways to reduce the effects of climate change. One way to do this is to focus on producing more plant-based foods instead of meat. This is because plant-based foods create less pollution than meat does. As a result, scientists are trying to find more protein sources that come from plants, like the ones found in rapeseed. Rapeseed is a type of plant that is mostly used to make edible oil, also known as canola oil. When oil is made from rapeseed, there is a part left over that has a lot of protein in it. This part is called press cake and is mostly made up of fibre, protein, and some leftover oil. Right now, rapeseed press cake is used as food for animals. People don't usually eat the protein from rapeseed because it can taste bitter and is hard for our bodies to use. Nevertheless, scientists from the Department of Food Technology, Engineering and Nutrition at Lund University have found a way to get the protein out of the press cake. This breakthrough opens up the possibility of incorporating this protein into food products, making it suitable for human consumption in the near future.

However, the latter has been successfully performed on a small scale, and the challenge now is not only to produce it on a larger scale but also to find the most optimal way to do it. That being said, the goal of this study was to improve the capacity of this process and get more protein from rapeseed press cake. To accomplish the former, the study was comprised of two steps, hereafter referred to as the first protein recovery step and the second protein recovery step. In the initial protein recovery process, the rapeseed press cake was milled to enable the separation of proteins from the cake in subsequent steps. The resulting powder was combined with water and mixed continuously for an hour while adjusting the pH to 7. Subsequently, the mixture was transferred to the decanter centrifuge, which is the essential machine for separating the proteins from the rapeseed press cake. This centrifuge operates continuously and separates the materials based on their density. During this step, two streams emerged from the centrifuge: a heavy phase containing residues and proteins that were unable to be separated from the rapeseed cake, and a liquid phase consisting of water and most of the proteins. That being said, different ways of operating the decanter centrifuge were tested. After conducting these experiments, it was discovered that the best way to extract protein from rapeseed press cake was to use a decanter centrifuge with an inlet flow rate of 60 L/h, 2000xg acceleration, 50 rpm differential speed, and a weir disc with a 68 mm inner diameter. These values were found to be the most optimal at this flow rate when compared to the other tested inlet flow rates, 40 and 80 L/h.

The previously obtained liquid phase is then treated with acid. This helped the proteins to settle down at the bottom by removing their electrical charge. The mixture was then separated into two parts by employing the decanter centrifuge; this time, the liquid phase is called supernatant, and the heavy phase is called precipitate. The precipitate contains most of the proteins. The same procedure was followed during the second protein recovery. Based on a thorough analysis, it was determined that the most suitable set of processing parameters for the decanter centrifuge during the second step of protein recovery were an inlet flow rate of 10 L/h, 4000xg acceleration, 6 rpm differential speed, and a 56 mm inner diameter weir disc. The latter enhanced the overall process capability without any adverse effects on critical factors such as the amount of protein obtained. It was also observed that during the second protein recovery step, a lot of precipitate was found to be stuck on the walls of the machine because of how sticky this solid material was. This solid material was important to study because it could affect how much protein could be recovered. Consequently, the study found that scraping equipment surfaces resulted in a significant increase in protein and the amount of protein recovered. To demonstrate, the protein recovery yield increased from 17% to 29% when scraping the surfaces of the equipment.

Table of contents

1. Background

1.1. Overview

Due to ethical, health, and environmental concerns, more customers are choosing plant-based foods instead of conventional animal-based counterparts [1]. As a result, there is an increasing interest in investigating proteins of plant origin; the most popular plant-based proteins within the scientific community and food industry include soy, pea, chickpea and oats [2]. Nevertheless, there are just a few studies on protein extraction from cold-pressed rapeseed cake, and a common aspect in all these studies is the need for more standardisation of methodologies, both for the extraction of the proteins and for describing their functionalities. The latter makes a comparison between studies unfeasible. Consequently, this study will contribute to developing the understanding of the field of plant-based protein extraction, specifically related to the proteins obtained from cold-pressed rapeseed press cake (RPC).

Moreover, it is crucial to be able to scale up the rapeseed protein recovery process because many of the studies that are currently available are based on laboratory-scale setups [2]. On the bright side, the research group from the Department of Food Technology, Engineering and Nutrition of Lund University has already been able to scale up from a laboratory scale; consequently, the present study aims to enhance the overall capacity of the process and the ability to recover protein to the greatest extent, resulting in higher protein content and protein recovery yield.

1.2. Rapeseed crop: Current market status

Rapeseed is the term primarily used in Europe to identify oil-bearing seeds of *Brassica napus* and *Brassica rapa*. Rapeseed may grow in various environmental conditions, although it thrives most effectively in colder climates. In 2013 global rapeseed production was estimated to be 72.67 million tons, with the top five producing nations, Canada (17.80 million tons), China (14.46 million tons), India (7.83 million tons), Germany (5.78 million tons), and France (4.37 million tons), accounting for 69.2% of the total production [3]. According to European authorities, in 2023, the world's rapeseed production is anticipated to reach a record of 86.9 million tons, with an increase of 18% on a yearly basis, supported by a particularly good crop from top-producing countries [4]. In addition, rapeseed holds a prominent position in the worldwide oilseed market, coming in second (~13.6%) to soybean (~55%) in terms of oilseed production and third in terms of vegetable oil consumption, after palm and soybean oil, not to mention that rapeseed is the second-largest feed protein source [3].

1.3. Industrial processing: Rapeseed oil extraction and its by-product

Rapeseed is primarily utilised to produce edible oils, which are extracted by the mechanical separation of oil and solids, i.e., screw pressing. Cold pressing and expeller pressing, followed by extraction by solvents, are the methods that are most frequently employed to produce rapeseed oil [1]. Furthermore, when rapeseed oil is extracted, a protein-rich by-product called press cake is produced due to mechanical action during the pressing. This organic material is currently utilized as a source of protein in animal feed [5]. To produce 1 kg of rapeseed oil, 3 kg of rapeseed is required, and the process also yields 2 kg of RPC as a by-product. [6] Cold pressing is given special attention not only because it is a more environmentally friendly method, i.e., no solvents are used, but also because proteins extracted from cold-pressed RPC have shown higher quality in terms of functionality. This might result from the oil extraction process where the RPC is exposed to milder temperatures (55-60°C) than during hot pressing (80-105°C) [7].

1.4. Protein and nutritional profile

RPC primarily consists of fibre (30%), protein (27-30%) and residual oil (20%). Salt-soluble 12S globulins (cruciferin) and water-soluble 2S albumins (napin), which together comprise up to 80% of the overall protein content, are the two primary proteins in RPC. Oil body proteins comprise the remaining fraction [1]. Given their excellent thermal stability, cruciferin and napin have denaturation temperatures of 91°C and 110°C, respectively [8]. Moreover, rapeseed proteins have a well-balanced amino acid profile with a significant amount of essential amino acids (> 400 mg/g protein), notably sulphur-containing amino acids in 1:1 ratios (such as methionine and cysteine) (40–49 mg/g protein). However, the fact that it contains glucosinolates, phytates, and phenolic compounds, such as sinapic acids and tannins, counterbalances the overall nutritional benefit. The presence of these antinutritional properties hinders the use of rapeseed protein in food applications due to the bitter taste and the creation of protein complexes that, alongside the seed hulls, reduce the bioavailability of nutrients [8].

1.5. Extraction methods

The most common protein recovery processes in the plant-protein field are the alkaline extraction method and the salt extraction method. Among these two, the predominantly reported method is the alkaline extraction of proteins followed by precipitation with a diluted acid. To obtain high-protein recovery yields, high alkaline pH values of 11-12 attained with sodium hydroxide (NaOH) are typically required. Thereafter, to recover protein, the extract's pH is adjusted to the point where minimum protein solubility is observed, i.e., isoelectric point (pI). Although alkali extraction has a high protein recovery yield, it often leads to the production of protein compounds with a dark brown to grey colour. This is caused by the oxidation of phenolic chemicals and protein-phenolic interactions that result in bitterness and other undesirable organoleptic characteristics [3].

On the other hand, considering this will be the approach employed, the salt extraction method is of great relevance to this study. In detail, the latter is based on the principle of salting-in. This allows both cations and anions to interact with the protein, causing a disruption of the attractive forces between protein molecules, resulting in an increased solubility [9]. Again, the extract’s pH is adjusted to the pI. Due to the milder processing conditions utilized in the latter method, the adverse effects encountered with the alkaline method are avoided.

1.6. Laboratory- and pilot-scale protein recovery: Benchtop centrifuge and decanter centrifuge

A benchtop centrifuge is a piece of machinery powered by an electric motor that rotates an object around a fixed axis while exerting force perpendicular to the axis. Due to their compact size, benchtop centrifuges are advantageous for laboratories with limited space [10]. When performing protein recovery procedures on a laboratory scale, batches of around 2 L of liquid are employed. To scale up the procedure, equipment with continuous separation capabilities, such as a decanter centrifuge, is required. A decanter centrifuge is an equipment which employs centrifugal force in an extended rotating bowl to continuously separate suspended solids from a liquid. Its ability to continuously discharge separated solids from the rotor via an axial screw conveyor sets it apart from other types of centrifuges, e.g., benchtop centrifuges. As shown in Figure 1, to create a scrolling effect, the screw conveyor rotates in the same direction as the bowl but at a relatively different speed.

Additional unique features of the decanter centrifuge involve a round bowl with a narrow end that revolves around a horizontal axis, solids are discharged from the narrow end, and the liquid phase is discharged from the broad end in a counter-current flow. The capacity of the equipment on semi- and pilot-scale is within the range of 20-80 L/h, whereas on the industrial scale is around 10 000 L/h. In the dairy industry, decanter centrifuges are often utilized for clarifying whey. They are also commonly used in plant-based beverage production and wastewater treatment applications to dewater solids and sludge [11].

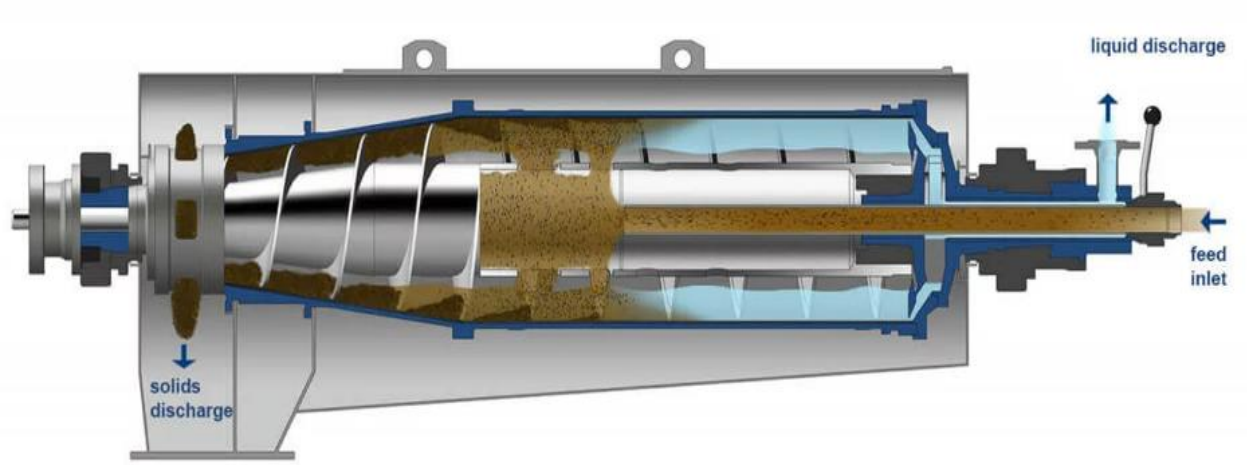

Figure 1. Sectional depiction of a decanter centrifuge [12].

1.7. Success factors of a decanter centrifuge

The processing parameters, such as the liquid flow rate of the inlet, differential speed, acceleration, and weir disc (WD) inner diameter dimensions, have a major impact on the performance of the separation process.

1.7.1. Inlet flow rate

It influences the length of time the solids reside in the decanter centrifuge and hence the extent to which the feed may be separated. High flow rates through the decanter result in fluids containing an excessive amount of solids. Low flow rates through the decanter, on the other hand, may cause material build-up in the screw conveyor or create blockage in the feed zone [13].

1.7.2. Differential speed

The gear unit produces a difference in speed between the decanter bowl and the decanter screw conveyor. This is necessary to convey the solid particles that accumulate on the inner diameter of the bowl employing the decanter screw conveyor. Thus, the amount of time the solid material stays in the bowl is dictated by the differential speed. Consequently, selecting the right differential speed helps to retain the solid in the bowl for the ideal period of time, resulting in optimal dewatering and dry matter content that can be achieved in the solids being discharged; it also aids in overload and blockage protection [14].

1.7.3. Acceleration

The acceleration value of the decanter centrifuge is directly related to the speed. The higher the rotational speed, the more efficient the separation [15].

1.7.4. Weir disc and pond depth

The pond depth is adjusted by changing the weir plates. Figure 2 illustrates that at a smaller WD inner diameter, the liquid volume and hence the time spent by the liquid in the decanter surges as the pond depth increases, i.e., deep pond. This increases the performance of the clarifying process. The drying area in the cone of the decanter bowl, on the other hand, is also decreased. As a result, the solid spend less time outside of the liquid. This implies that the discharged solid is moister, and the machine's dewatering efficiency is diminished. The opposite happens at larger WD inner diameter as the pond depth decreases, i.e., shallow pond [14].

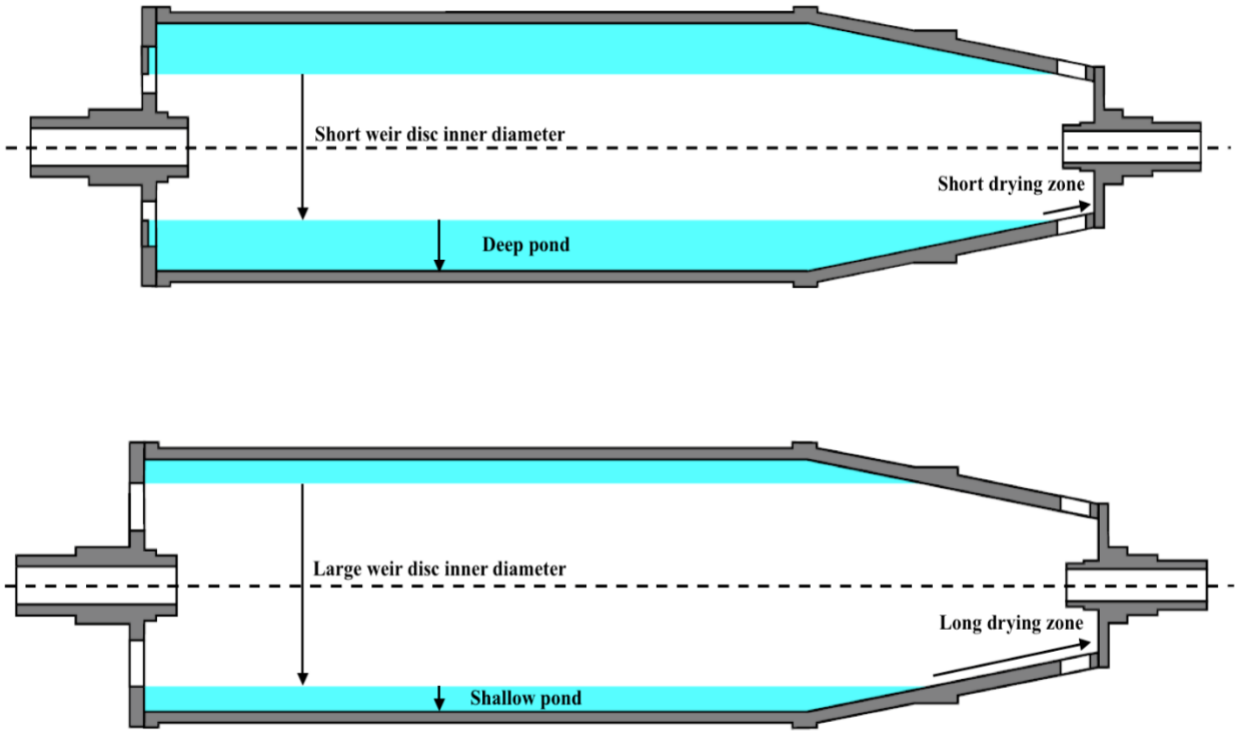

Figure 2. Modified illustration of WD inner diameter and its effect on the pond depth [16].

1.8. Aim

The aim of the study was to study the pilot-scale process of protein extraction from cold-pressed rapeseed cake employing the salt extraction method and a decanter centrifuge as the first separation step, understanding its effect on the dry matter and protein content of the LLP.

Additionally, the study aimed to investigate the feasibility of performing the second separation step on a pilot scale by first employing the isoelectric precipitation method and, thereafter, a decanter centrifuge. The latter once the first separation step has been optimised.

2. Material and methods

2.1. Resources

The experimental work, including physicochemical analyses, was carried out at the pilot hall and laboratory rooms of the Department of food technology, engineering, and Nutrition at the Faculty of Engineering of Lund University.

2.2. Materials and chemicals

Cold-pressed RPC from Gunnarshögs Jordbruks AB, located in the south of Sweden, was employed in the two-step protein recovery trials conducted throughout the period of the research. Their oil manufacturing plant is currently producing cold-pressed rapeseed oil employing screw pressing at mild processing temperatures, i.e., 37° C. The temperature of the RPC at the outlet of the equipment oscillates between 55-60 °C [6]. Chemicals used for pH adjustment, Sodium hydroxide (NaOH) and Citric acid, were acquired from Merck (Darmstadt, Germany), whereas aspartic acid was purchased from Thermo Electron (Milan, Italy).

2.3. Two-step protein recovery from cold-pressed rapeseed press cake

The present research employed the modified 2-step protein-recovery methodology from Östbring et al. and Ekwall to recover protein from RPC, see Figure 3 [7, 17]

2.3.1. First protein recovery step: Extraction of protein from rapeseed cake

To begin with, 10 kg of RPC were ground in a knife mill (Robot coupe, R 5 Plus, Burgundy, France) for 3 minutes to yield a very fine powder. Due to the capacity of the equipment, the grinding was conducted in batches of 1 kg of RPC each. Thereafter, the total amount of ground powder and 1.26 kg of NaCl (0.25 M) were dispersed in 90 kg of tap water at around 10°C (RPC-water ratio of 1:9 w/w) in a 150 L stirred tank (Wedholms, DH 653, Nyköping, Sweden), and the pH was manually adjusted to 7 by employing a solution of 1M NaOH. Under continuous stirring, the pH of the dispersion was kept constant at 7 for one hour using a pH controller (Hanna, HI9910, Limena, Italy) with a dosing pump (Watson Marlow, 120U, Massachusetts, United States). Due to the large volume in the tank, two three-bladed propeller stirrers (IKA, RW 28 digital, Staufen, Germany) and (IKA, Eurostar power control-visc, Staufen, Germany) were needed to achieve a continuous stirring under 400 rpm and 1100 rpm, respectively.

Subsequently, a peristaltic pump (Masterflex Easy-load Model 77200-62, Cole-Parmer, Vernon Hills, IL, USA) was employed to pump the dispersion into the decanter (Decanter centrifuge MD80, Lemitec , Berlin, Germany). In order to assess the overall impact of the processing parameters on the dry matter and protein content at this point, the decanter centrifuge and peristaltic pump were operated at different settings; these 48 evaluated combinations are shown in Table 1. Samples from the light liquid phase (LLP) and the spent solid fraction (SSF) were collected every 7 minutes at each processing setting and stored at −18 °C for further analysis. The reasoning for the latter sampling frequency is based on the residence time provided by the equipment's manufacturer, in which the residence time of the product in the bowl at an inlet flow rate of 20 L/h is between 40 and 75 seconds depending on the WD being used [18]. To ensure a representative sample, it was determined that 7 minutes would be allotted. This allowed the equipment to stabilize and produce a representative LLP and SSF sample before shifting on to the next set of processing parameters.

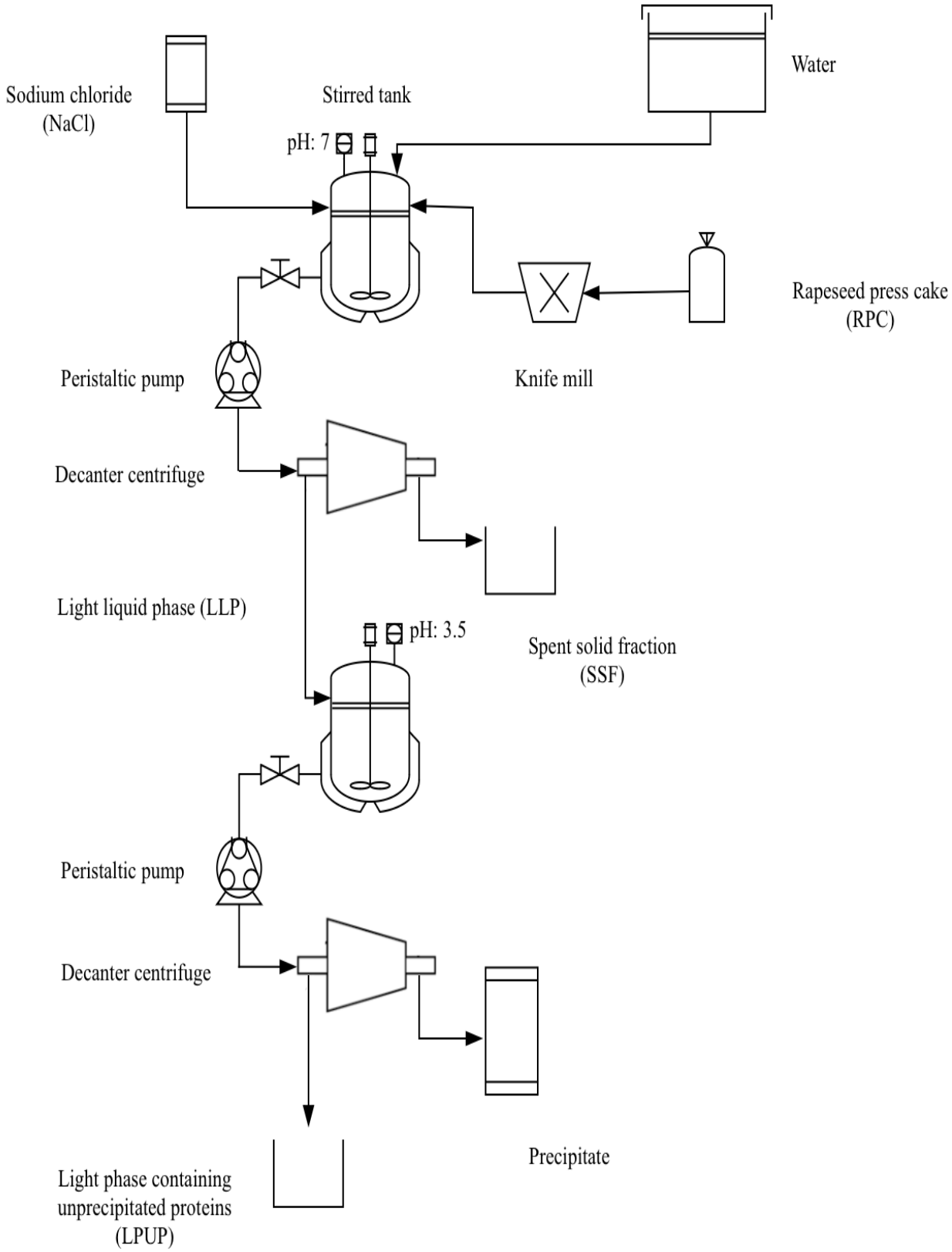

Figure 3. Employed two-step rapeseed protein recovery process diagram.

Table 1. Overview of the combination of processing parameters that were assessed during the first protein recovery step.

Inlet flow rate (L/h)	WD inner diameter (mm)	Differential speed (rpm)	Acceleration (g)
40	56	10, 50	1000
			2000
			3000
			4000
	68	10, 50	1000
			2000
			3000
			4000
60	56	10, 50	1000
			2000
			3000
			4000
	68	10, 50	1000
			2000
			3000
			4000
80	56	10, 50	1000
			2000
			3000
			4000
	68	10, 50	1000
			2000
			3000
			4000

In order to simplify sample handling and data analysis, the study followed the nomenclature system described in Table A in the appendix. To begin with, the reference sample for comparison was called "Benchmark", and the processing parameters for this sample were based on a study by Ahlström et al. in 2022. The latter aimed to optimize protein recovery from RPC in a pilot scale set-up by using an alkaline extraction method at pH 10.5, followed by a decanter centrifuge operating under the standard processing conditions (20 L/h, 2000xg, 10 rpm, and 56mm) [1]. These settings were applied to the present study employing the salt extraction method. Consequently, it provided a foundation for the present research on RPC protein recovery, particularly in terms of process optimization, since it enabled comparison between samples. Regarding the nomenclature system, the 5-digit numbers represent various combinations of processing parameters that were evaluated during the initial protein recovery stage. To explain, the first two digits from left to right indicate the inlet feed rate (20, 40 and 60 L/h), followed by a single digit for acceleration (1000, 2000, 3000 and 4000xg), the next single digit for the differential speed (10 and 50 rpm), and finally, the last digit indicates the inner diameter of the WD (56mm and 68mm). For instance, 40115 stands for 40 L/h, 1000xg, 10 rpm and 56 mm. Prior to proceeding to the second stage of the protein recovery process, it was decided to employ the following set of processing parameters based on the performance evaluation in section 3.1: 60 L/h inlet feed rate; 2000xg acceleration; 50 rpm differential speed; and 68 mm WD.

2.3.2. Second protein recovery step: Isoelectric precipitation of protein from rapeseed cake

The second protein recovery step was performed by first adjusting the LLP pH to 3.5 using citric acid and a stirrer at 600 rpm (IKA Eurostar power control-visc, Staufen, Germany). The buckets containing LLP were stored overnight in the fridge at 10 ◦C prior to further processing. Thereafter a peristaltic pump (Masterflex Easy-load Model 77200-62, Cole-Parmer, Vernon Hills, IL, USA) was employed to pump the LLP into the decanter (Decanter centrifuge MD80, Lemitec, Berlin, Germany). To prepare for each trial, the LLP was stirred at 600 rpm for 5 minutes. Afterwards, the pH was checked and adjusted to the pI value of 3.5 if necessary. In order to assess the overall impact of the processing parameters on the protein recovery yield at this point, the decanter centrifuge and peristaltic pump were operated at different settings; these evaluated combinations are shown in Table 2.

The settings were selected after optimizing the initial protein recovery process and conducting a screening procedure based on modified research conducted by Ekwall in 2021. The aim of her study was to optimize the second separation step by using the same protein recovery strategy, which involves salt extraction, isoelectric precipitation, and a decanter centrifuge, to effectively precipitate proteins from RPC [17]. In order to obtain reliable results, it was crucial to run each set of processing parameters for 1.5 hours. This timeframe was necessary to compare the protein recovery yield for 3 different inlet flow rates within a specific time period. A shorter duration would not have been sufficient as it takes between 30 and 60 minutes (depending on the inlet flow rate) for the precipitate to be discharged from the decanter centrifuge, which is essential for accurate results. On the other hand, exceeding a run time of 1.5 hours would have had negative consequences on the execution of the project. This is because of the time constraints and the limited availability of equipment later on. The amount of LLP pumped into the decanter centrifuge varied depending on the inlet flow rate being tested. At a rate of 5 L/h, 7.5 L of LLP were pumped. At 10 L/h, 15 L of LLP were pumped. And at 15 L/h, 22.5 L of LLP were pumped. Samples from the light phase containing unprecipitated proteins (LPUP) and precipitate were collected at the completion of each run and stored at −18 °C for further analysis.

Table 2. Combination of processing parameters that were assessed during the second protein recovery step.

Inlet flow rate (L/h)	WD inner diameter (mm)	Differential speed (rpm)	Acceleration (g)
5	56	6	4000
10			
15			

2.4. Process performance evaluation

Three key values, extraction coefficient, precipitation coefficient, and protein recovery yield, were calculated to quantify the effect that processing parameters have on the performance of the process. For this reason, all weights were noted during the execution of the experiments.

2.4.1. Extraction coefficient

It indicates the extent to which the protein present in the RPC was solubilized in the aqueous phase during the first protein recovery step [19].

$$Extraction\ coefficient\ (\%): \left(\frac{Mass\ of\ protein\ in\ the\ LLP\ (g)}{Mass\ of\ protein\ in\ the\ RPC\ (g)}\right) * 100 \quad (1)$$

2.4.2. Precipitation coefficient

It indicates the extent to which the protein present in the LLP was precipitated at a pH of 3.5 during the second protein recovery [19].

$$Precipitation\ coefficient\ (\%): \left(\frac{Mass\ of\ protein\ in\ \ precipitate\ (g)}{Mass\ of\ protein\ in\ the\ LLP\ (g)}\right) * 100 \quad (2)$$

2.4.3. Protein recovery yield

It indicates the amount of protein present in the RPC that was recovered in the precipitate [1]

$$Protein\ recovery\ yield\ (\%): \left(\frac{Mass\ of\ protein\ in\ the\ precipitate\ (g)}{Mass\ of\ protein\ in\ the\ RPC\ (g)}\right) * 100 \quad (3)$$

2.5. Spin test

In a spin test, test tubes containing sample fluid are spun in a benchtop centrifuge to produce performance data [20]. For this reason, spin tests were performed on the LLP samples collected at the first protein recovery step with the aim of obtaining an estimate of the process performance. The spin test was performed by adding 10 mL of LLP to a graduated centrifuge tube. These tubes were then spun in a benchtop centrifuge (Beckman Coulter, Avanti J-15R Centrifuge, Brea, CA, USA) for 10 min at 4700xg and 20 °C. At the conclusion, the amount of solids was determined using the scale that the tubes provided (1-10 % v/v).

2.6. Proximate analysis

The proximate analysis consisted of dry matter and protein content analyses of samples taken from RPC, LLP and SSF at the first protein recovery step and LPUP and precipitate at the second protein recovery step.

2.6.1. Dry matter content

The standardized method of analysis (AOAC 934.01) was employed to determine the dry matter content of the RPC. In this case, the fine powder was placed, in triplicate, in a convection oven (Termaks, Bergen, Norway) at 103°C for a minimum of 16 hours in order to achieve a constant weight. On the other hand, freeze-drying was employed to facilitate handling due to a large number of samples taken from LLP, SSF, LPUP and precipitate. For the latter, the samples were placed, in duplicate, in a freeze dryer (Labconco Lyph Lock 18, Kansas City, MO, USA) for four days. To guarantee that all water had been removed, all the samples were subsequently placed in a desiccator for 1 day. The dry matter content was calculated using the following equation:

$$Dry\ matter\ content\ (\%): \left(1 - \frac{Sample\ before\ drying\ (g) - Sample\ after\ drying\ (g)}{Sample\ before\ drying\ (g)}\right) * 100 \quad (4)$$

2.6.2. Protein content

The Dumas method (AOAC 990.03) (Thermo Electron Corp., Flash EA, 1112 Series, Waltham, MA, USA) was employed to determine the protein content of the samples after drying. With this in mind, 25-30 mg of the sample, in duplicate, was placed into a thin disc for analysis. Aspartic acid served as the standard and air as the blank. A nitrogen-to-protein conversion factor of 6.25 was used.

2.7. Statistical analysis

In order to gauge the extent of difference between two distinct sets of data, a two-tailed student's t-test was employed. Specifically, this test was utilized to analyse the LPP dry matter and protein content values that had been previously measured in duplicate during the initial protein recovery process. To achieve statistical significance, any probability values that were below 5% ($p \leq 0.05$) were considered significant. The latter was performed by employing Microsoft Excel.

3. Results and discussion

Each batch was produced in accordance with the steps outlined in the Material and Methods section. The LLP was assessed in terms of spin test, dry matter, and rapeseed protein content on a dry basis (db %) under several conditions in order to ascertain the most optimal processing parameters for the first rapeseed protein recovery step. With this in mind, samples from LLP and SSF were collected every 7 minutes at each processing setting and stored at −18 ◦C for further analysis. Once a significant number of samples were taken, they were defrosted and prepared for spin test, dry matter and protein content analysis. Tables B and C in the appendix provide the raw data for the latter.

3.1. Optimization of the first protein recovery step

3.1.1. Process performance evaluation at 1000xg

A spin test was conducted on LLP samples to obtain a rough estimate of how the process would perform and thereafter correlate these results with dry matter and protein content. As shown in Figure 4, samples taken from runs using the 68 mm WD had greater solid content (%v/v) than samples taken from runs using the 56 mm WD. This is because a larger WD inner diameter produces a shallower pond that results in a decrease in the volume of the product contained in the bowl. At the same time, this decrease leads to a shorter residence time. The last-mentioned outcomes exposed to the centrifugal force restrict the solid separation. As a result, the generated LLP has higher solids content.

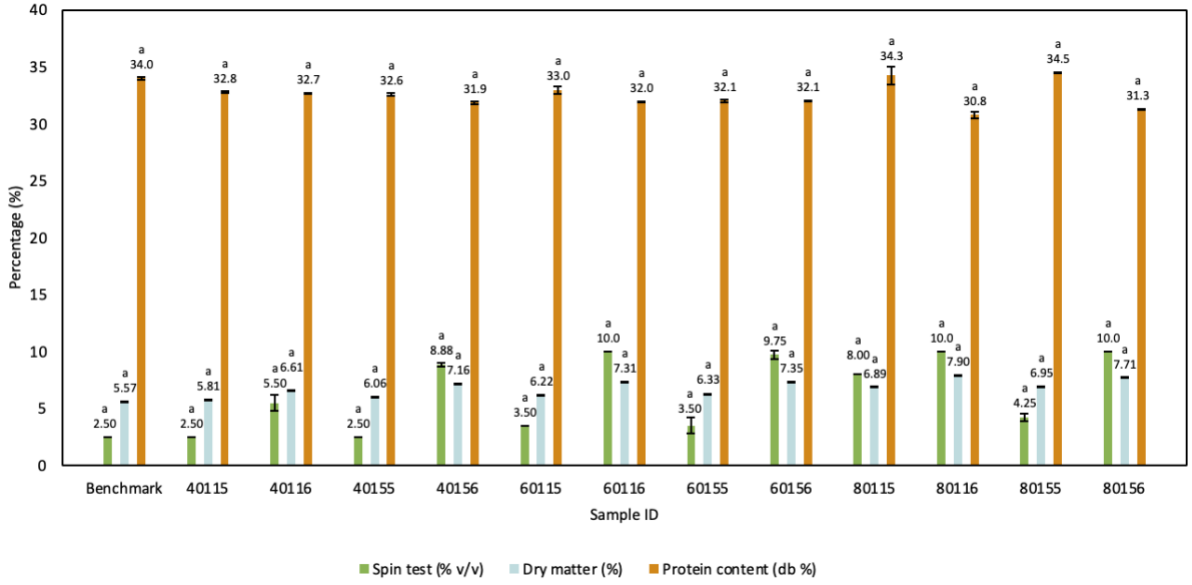

Figure 4. Spin test (% v/v), dry matter (%), and protein content (db %) of the LLP for different sets of processing parameters at 1000xg. Different letters indicate a significant difference ($p \leq 0.05$).

While the spin test provided a preliminary understanding, it was not entirely precise. The reason for this inaccuracy was due to the limited measurement scale of the centrifuge tubes employed. Consequently, some samples with a high concentration of solids could not be accurately measured due to the tubes' capacity constraints (1-10 %v/v), e.g., samples shown in Figure 4 with values of 10 %v/v. Given these points, it was decided not to rely on the spin test results. It is clear that the dry matter content followed the same pattern as the spin test findings, as a shallow pond leads to a less clarified LLP.

It is important to note that the results obtained from samples during runs using the 68 mm WD are slightly larger than the counterpart; these values are within the range of 5.81-7.90%. The latter suggests that even if the values of the dry matter content fluctuate based on different sets of processing parameters, based on the statistical analysis, the deviation is not substantial. In like manner, higher differential speed yielded LLP with higher dry matter content. To explain further, a larger difference in speed between the bowl and screw conveyor led to a shorter residence time. This not only resulted in less effective separation of solids but also decreased the capacity for SSF drying, resulting in a higher level of water in the SSF product, which is important in downstream processing. It was found that the LLP dry matter content was the highest at 80116, with a value of 7.90 ± 0.0%. However, the protein content was low at 30.8 ± 0.3%. On the other hand, the lowest dry matter content was discovered at 40115, with a value of 5.81 ± 0.0%. But it had a high protein content of 32.8 ± 0.1%.

As seen in Figure 4, when the inlet flow rate was raised, the protein content also increased. These findings pertain solely to LLP samples obtained from runs that utilized the 56 mm WD, where the protein content ranged from 32.1% to 34.5%. However, when utilizing the 68mm WD, the protein content in LLP samples decreased as the inlet flow rate increased, with values ranging from 30.8% to 32.7%. A shallow pond coupled with a higher inlet flow rate led to a shorter residence time and a hindered separation performance. However, when a shorter WD inner diameter was used (Deep pond), the opposite effect occurred. Lastly, it was found that the LLP protein content was highest at 80115, yielding 34.3 ± 0.0%. Conversely, the lowest LLP protein content was observed at 80116, with a value of 30.8 ± 0.3%.

3.1.2. Process performance evaluation at 2000xg

Regarding the spin test, samples from runs with a 68 mm WD had more solid content than those with a 56 mm WD due to the shallower pond created by the larger disc, leading to decreased product volume and shorter residence time. This resulted in lower solid separation and higher solids content in the LLP. Furthermore, the dry matter content showed a similar trend to the previous evaluation at 1000xg; see Figure 5. However, due to the product being subjected to larger centrifugal forces of 2000xg, the dry matter content slightly decreased compared to the previous set of parameters. This indicates that solid separation was more efficient at higher acceleration values, removing not only unwanted solids but also other components found in RPC.

Therefore, it was determined that the LLP dry matter content was highest at 80256, with a value of 7.02 ± 0.1%. The lowest content was found at 40215, with a value of 5.62 ± 0.0%. In addition, it can be seen that several LLP sample data are unavailable. This occurred when processing settings were set at low differential speed (10 rpm) and high inlet flow rates (40 L/h, 60 L/h, and 80 L/h), causing an unexpected increase of solids inside the bowl and a subsequent excessive torque in the screw conveyor. As a safety measure, the automatic decanter shutdown feature was triggered due to overload. Consequently, it was not possible to collect some LLP samples at the end of the 7-minute period. The former was avoided when the differential speed was set to 50 rpm. This resulted in a shorter residence time as the solids were conveyed through the screw conveyor at a faster rate.

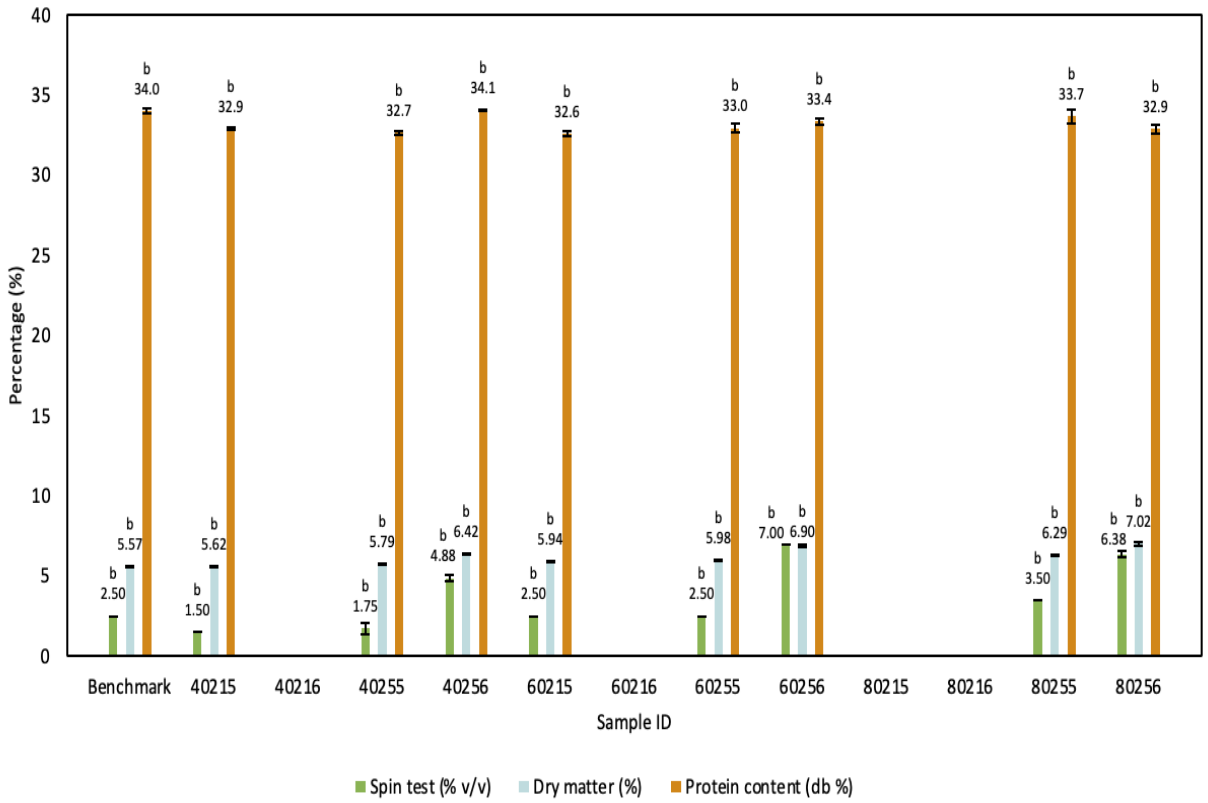

Figure 5. Spin test (% v/v), dry matter (%), and protein content (db %) of the LLP for different sets of processing parameters at 2000xg. Different letters indicate a significant difference ($p \leq 0.05$).

The results of the protein content at 2000xg were also consistent with the previous evaluation at 1000xg. When using the 56 mm WD, higher inlet flow rates led to an increase in protein content in the LLP samples. The percentage of protein content ranged from 32.6% to 33.7%. However, when using the 68mm WD, a higher inlet flow rate led to a decrease in the protein content in the LLP samples, with values ranging from 34.1% to 32.9%. Based on these findings, the highest measurement of LLP protein content was recorded at 40256, reaching 34.1 ± 0.0%. On the other hand, the lowest LLP protein content was observed at 60215, with a recorded value of 32.6 ± 0.2%.

3.1.3. Process performance evaluation at 3000xg

In the current evaluation, the spin test and dry matter content followed a trend similar to the previous ones, as shown in Figure 6. However, the product was exposed to higher centrifugal forces of 3000xg, resulting in a slight decrease in the dry matter content compared to the previous set of processing parameters. This suggests that solid separation was more effective at higher acceleration values. It was concluded that the LLP had the highest dry matter content at 80356, with a value of 6.63 ± 0.0%. Conversely, the lowest content was observed at 40315, with a value of 5.65 ± 0.0%. It can also be seen that several LLP sample data are unavailable since the automatic decanter shutdown feature was activated due to an overload in the screw conveyor. However, this issue was not experienced at a differential speed of 50 rpm because the solids were transported through the screw conveyor at a faster rate (less torque needed), resulting in a shorter residence time for the solids.

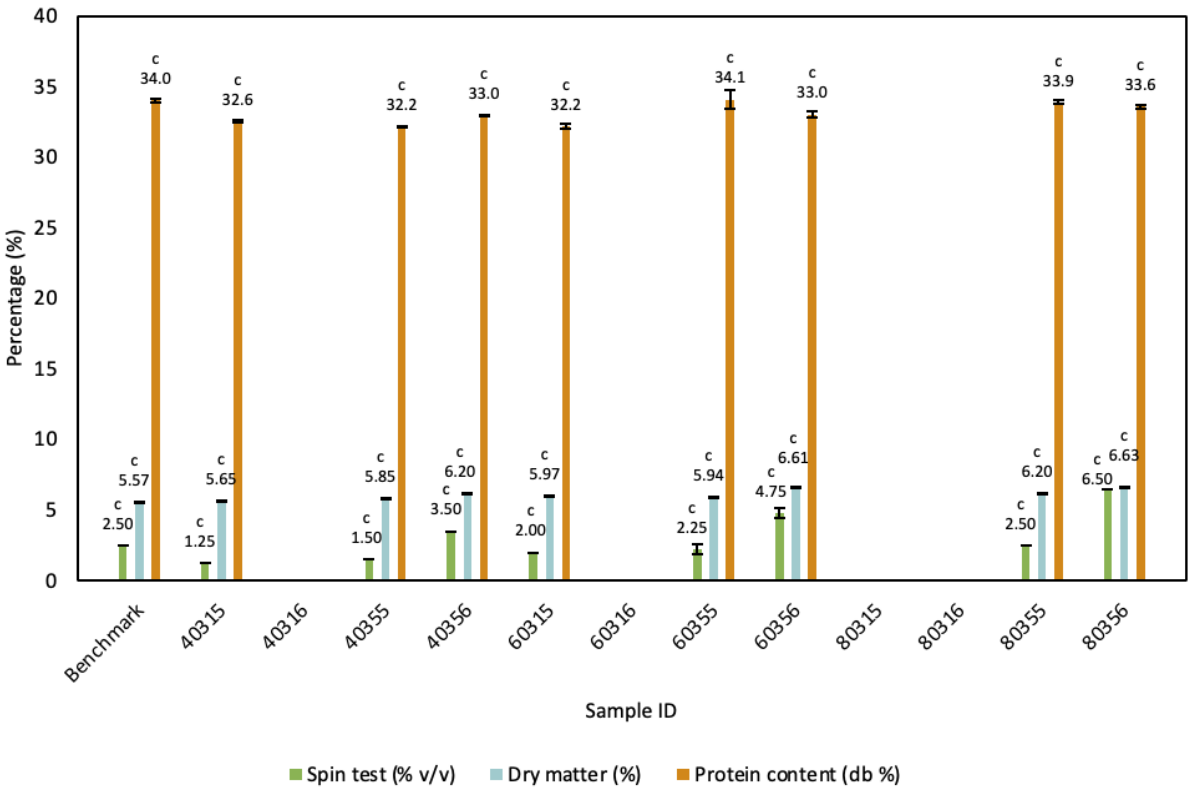

Figure 6. Spin test (% v/v), dry matter (%), and protein content (db %) of the LLP for different sets of processing parameters at 3000xg. Different letters indicate a significant difference ($p \leq 0.05$).

The results presented in Figure 6 demonstrated that increasing the inlet flow rate also results in a slight increase in protein content. This trend was observed in both LLP samples obtained from runs that used the 56mm and 68mm WD. The protein content ranged from 32.2% to 34.1% for the 56mm disc and 33.0% to 33.6% for the 68mm disc. In detail, the LLP protein content peaked at 60355, registering a value of 34.1 ± 0.7%. Conversely, the lowest LLP protein content was observed at 40355, with a value of 32.2 ± 0.0%. These new findings go against what was previously observed in the evaluations conducted at 1000xg and 2000xg. Even though a shallow pond (Large WD inner diameter) coupled with a higher inlet flow rate led to a shorter residence time and a hindered separation performance. It is possible that acceleration plays a more significant role in determining the overall performance of the process.

3.1.4. Process performance evaluation at 4000xg

As can be seen in figure 7, the evaluation of process performance at 4000xg yields similar results to the previous evaluation at 3000xg. Namely, the LLP dry matter content was highest at 80456, with a value of 6.55 ± 0.0%. The lowest content was found at 40415, with a value of 5.42 ± 0.0%. Whereas the highest measurement of LLP protein content was recorded at 80456, reaching 34.0 ± 0.1%. On the other hand, the lowest LLP protein content was observed at 40456, with a recorded value of 32.5 ± 0.1%. Therefore, there is no need to discuss it further since the same outcome can be achieved by employing a variety of processing parameters at 3000xg. While energy consumption is not within the scope of this research, it's worth noting that running the equipment at such high acceleration can be quite energy intensive. Moreover, the high-pitched noise produced during the operation may not be ideal in a pilot-scale environment due to occupational noise exposure.

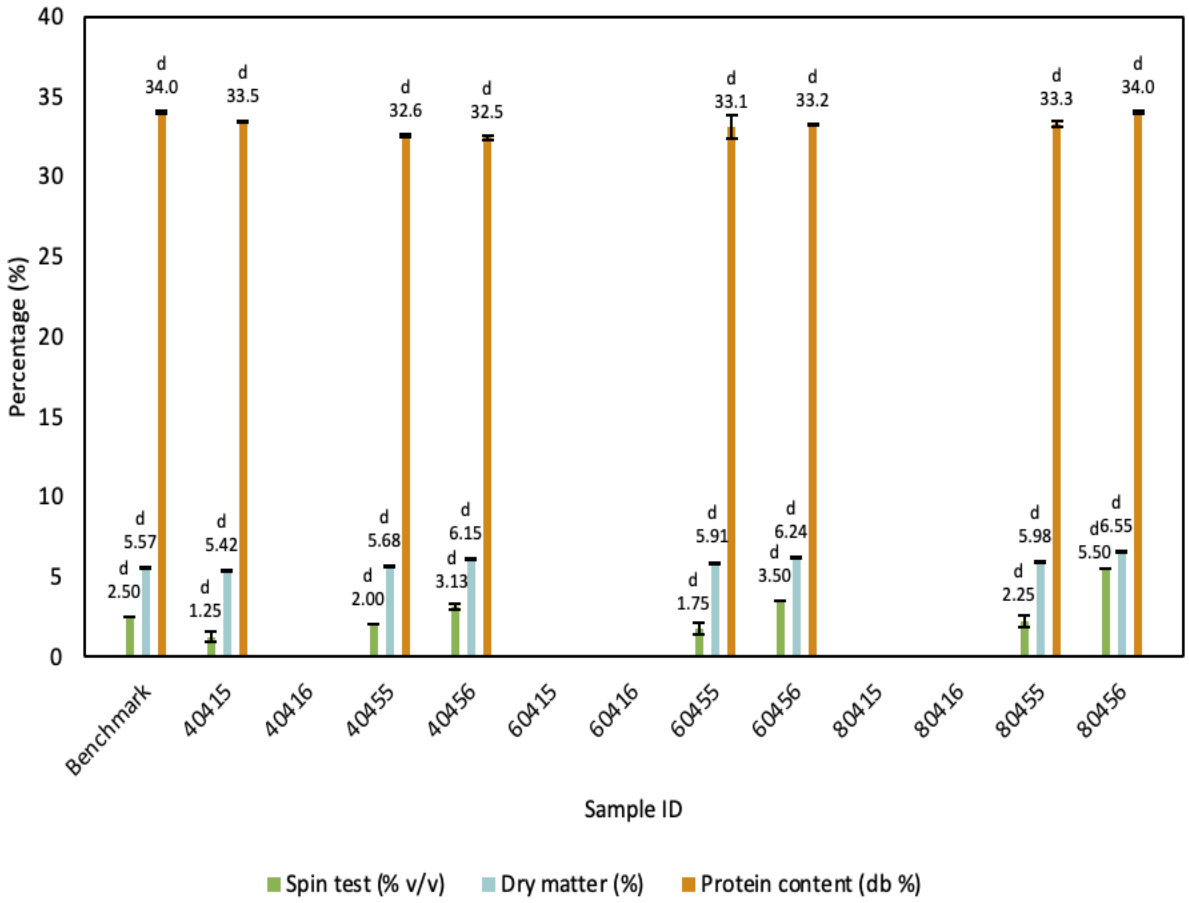

Figure 7. Spin test, dry matter, and protein content (db %) of the LLP for different sets of processing parameters at 4000xg. Different letters indicate a significant difference ($p \leq 0.05$).

Again, LLP sample data was unavailable due to the automatic decanter shutdown feature being activated from an overload in the screw conveyor. This issue did not occur at a differential speed of 50 rpm because the solids were transported faster, resulting in shorter residence time for the solids. For this reason, selecting the appropriate differential speed is essential to achieve optimal process performance, particularly in applications where there is a high concentration of solid material at the inlet. Thus, the handling capacity of the equipment is significantly influenced by this factor. It is also crucial to note the importance of properly stirring the dispersion during the extraction process. If this step is not performed correctly, solid sedimentation is likely to occur. Therefore, pumping a well-mixed liquid into the decanter can enhance the performance of the separation. The above factors, combined with operating the equipment at its maximum capacity, resulted in the decision to avoid using high acceleration during the initial protein recovery step. This was decided to ensure that the process remained safe and efficient.

3.1.5. Optimization of the first protein recovery step

Prior to proceeding to the second stage of the methodology, it was decided to employ the following set of processing parameters based on the latter performance evaluation: 60 L/h inlet feed rate; 2000xg acceleration; 50 rpm differential speed; and 68 mm WD, as the latter yielded 6.9 ± 0.0% and 33.4 ± 0.2% dry matter and protein content, respectively. The logic is consistent with the aim of the project, which is to increase overall process capacity without compromising dry matter and protein recovery yield. In fact, it was seen that at an inlet flow rate of 60 L/h, the combination of dry matter and protein content values of

LLP was found to be the most optimal compared to those obtained at 40L/h and 80L/h.In addition, at a higher flow rate, i.e., 80 L/h, the equipment triggered the automatic shut-down safety feature due to an overload caused by the significant amount of solids and, consequently, excessive torque in the screw conveyor. As a result, 80L/h was not suitable for this application. These results were also compared with the benchmark values previously obtained during the study at a pilot scale, 5.57 ± 0.0 % dry matter content and 34.0 ± 0.1% protein content. It is important to note that the dry matter, in this case, was lower since laboratory-scale centrifuges are way more efficient in separating components from the LLP than a pilot-scale decanter centrifuge, for instance, non-protein components.

3.2. Optimization of the second protein recovery step

3.2.1. Screening procedure

Following the optimization of the initial protein recovery step, a thorough screening procedure was implemented to build upon the modified research conducted by Ekwall in 2021; in her study, the objective was to enhance the second separation step to effectively precipitate proteins from RPC by employing the same protein recovery methodology, i.e., salt extraction method followed by isoelectric precipitation and a decanter centrifuge [17]. The latter was limited to a low inlet flow rate (5 L/h), whereas the present study focused on improving the overall capacity of the process; hence higher inlet flow rates (10 and 15 L/h) alongside different differential speeds (4, 6 and 8 rpm) at a fixed acceleration value of 4000xg were tested during the screening procedure. When conducting experiments on various differential speeds while keeping the inlet flow rate constant at 5 L/h, see Table 3, it was observed that the driest precipitate was obtained at 4 rpm, which had the highest dry matter content of 30.6 ± 0.0%. However, this differential speed resulted in the lowest protein content of 52.7 ± 1.0% when compared to 6 and 8 rpm.

Table 3. Dry matter and protein content of the precipitate during the screening procedure of the second protein recovery step at 5L/h, 4000xg and a WD inner diameter of 56mm. Different letters in each row indicate a significant difference ($p < 0.05$).

Differential speed	Dry matter (%)	Protein content (db %)
4	30.6 ± 0.0 [a]	52.7 ± 1.0 [a]
6	28.7 ± 0.2 [a]	56.7 ± 1.3 [a]
8	27.3 ± 0.1 [a]	53.4 ± 0.2 [a]

On the other hand, the best outcome in terms of the protein content of the precipitate was observed when the differential speed was set to 6 rpm, not only because it yielded the highest protein content (56.7 ± 1.3 %) but also because no material build-up was noted on the surface of the screw conveyor. In addition, according to the statistical analysis, values did not significantly differ from one another with respect to the dry matter composition of the precipitate. As a result, it was decided to solely test the inlet flow rates (5, 10 and 15 L/h) in the subsequent stage, leaving acceleration, differential speed, and WD set at 4000xg, 6 rpm, and 56 mm, respectively (see next section).

3.2.2. Optimization of the second protein recovery step

After deciding the processing parameters to be employed at this step, the run time per each set of processing parameters was set to 1.5 h to standardize and allow comparison between processing conditions. Samples from both LPUP and precipitate were taken to have a better understanding of the process. As a result, it can be seen in Table 4, that the highest protein content for the precipitate was observed at the lowest inlet flow rate (51.5 ± 0.4 %); again, it is worth mentioning that according to the statistical analysis, the difference

between protein content values was not significant, since 10 L/h and 15 L/h yielded 51.3 ± 0.3 % and 49.3± 0.2% respectively. LPUP, on the other hand, yielded the highest at an inlet flow rate of 15L/h. The former suggests that separation performance at the highest flow rates hinders the process performance to a certain extent; this is because the goal at this stage is to recover as much protein as possible in the precipitate and not in the LPUP. It was also noted that as the protein content in the precipitate decreased, the protein content in the LPUP increased.

Table 4. Dry matter and protein content of LPUP and precipitate at 4000xg, 6 rpm and 56 mm varying the inlet flow rates during the second protein recovery step. Different letters in each row indicate a significant difference ($p \leq 0.05$).

Inlet flow rate (L/h)	Sample	Dry matter (%)	Protein content (db %)
5	LPUP	6.29 ± 0.0 [a]	18.3 ± 0.3 [a]
	Precipitate	27.2 ± 0.0 [a]	51.5 ± 0.4 [a]
10	LPUP	6.79 ± 0.0 [a]	17.9 ± 0.2 [a]
	Precipitate	26.7 ± 0.0 [a]	51.3 ± 0.3 [a]
15	LPUP	6.97 ± 0.0 [a]	20.9 ± 0.1 [a]
	Precipitate	25.5 ± 0.0 [a]	49.3 ± 0.2 [a]

In addition, dry matter content in the precipitate was highest at 5L/h (27.2 ± 0.0%), and it decreased as the inlet flow rate increased to 10 L/h (26.7 ± 0.0%) and 15 L/h (25.5 ± 0.0%). The reason for the wetter precipitate and reduced dewatering efficiency of the equipment was mainly due to the increased inlet flow rate; this is to say that by increasing the inlet flow rate, the length of time the solids resided in the equipment was reduced, having a direct impact on the extent to which the inlet was separated, coupled with the use of a 56mm WD, which led to a deeper pond and a shorter drying zone in the bowl's conical section. As a result, the solids spent less time outside of the liquid. Dry matter content in the LPUP is quite similar at the three different assessed inlet flow rates. Indeed, it can be seen that the standard deviation is either zero or quite low as well. It should be highlighted that the screw conveyor did not experience any material build-up or feed zone blockage, even at higher inlet flow rates. This indicates that the processing parameters that were previously chosen were effective in ensuring the operability of the equipment. It is important to point out that the decision regarding the processing parameters for optimizing the second protein recovery step was based on the previous results coupled with the protein recovery yield, which will be explained in the following section.

3.3. Process performance evaluation

To quantify the impact of processing parameters on the process performance, three values were calculated: extraction coefficient, precipitation coefficient, and protein recovery yield.

3.3.1. Extraction coefficient

The extraction coefficient evaluated the performance of the first protein recovery step under pH 7 and 0.25 M NaCl and the employed set of processing parameters: 60 L/h inlet feed rate; 2000xg acceleration; 50 rpm differential speed; and 68 mm WD. The RPC and LLP quantities shown in Table 5 were obtained from a 100 kg batch, i.e., 10 kg RPC and 90 kg water. In addition, the mass of protein was calculated using the dry matter and protein content results.

Table 5. Extraction coefficient.

Material	Quantity (g)	Mass of protein (g)	Extraction coefficient (%)
RPC	10000	2800	61
LLP	70000	1700	

The obtained protein extraction coefficient of 61% is deemed satisfactory. It is important to note that not all proteins in the RPC could be solubilized during the extraction process, owing to the variations in protein properties, including protein structure and amino acid composition, which can affect their interactions. In addition, these interactions are also hindered due to interference from non-protein components. Protein solubility can also be affected by environmental factors like pH and salt concentration. The distribution and net charge of a protein are influenced by the pH of a solution. In general, proteins tend to be more soluble in increased pH environments due to the presence of charges with equal charge. Alkaline pH environments create a repulsive force between molecules, leading to a greater solubility [21]. Added to the latter, the employed salt extraction methodology, salt ions strengthened the electrostatic repulsive intermolecular protein-protein interactions leading to an increase of protein solubility (salting in) [9].

In this study, the aim was to extract proteins using a gentler approach. By employing a pH of 7, the number of solubilized proteins was reduced, leading to a lower extraction coefficient in comparison to previous studies that employed the alkaline extraction methodology with pH values around 10-12. Supporting this view, Rodrigues et al. found in 2017 that the protein extraction coefficient from RPC can be improved by pre-treating the material with phytase combined with alkaline extraction in a laboratory setup. The optimal conditions for protein solubilization were found to be at 75°C and pH 12.5 for 60 minutes, resulting in a protein extraction coefficient of 72.1%. This is significantly higher than the yield obtained from alkaline extraction alone, which was only 51.3%. Temperature and pH were found to be critical factors in the protein extraction process from defatted RPC [22]. Given these points, pH 7 was still deemed appropriate and, in combination with the addition of NaCl, yielded a 61% extraction coefficient.

In 2018 Fetzer et al. employed the salt extraction method with 0.25 M NaCl to compare protein extraction from cold-pressed and pre-pressed RPC. The results showed that protein extraction was significantly higher in cold-pressed RPC, with yields of up to 52.3%, compared to pre-pressed RPC, with yields of up to 36.7%, under mild pH conditions (pH 5.7-9). The study also found that protein extraction was improved under strong alkaline conditions and with the use of Protease A-01. When the pH was increased to 11 and 12, yields increased to 59.5% for cold-pressed RPC and 43.9% for pre-pressed RPC [23].

3.3.2. Precipitation coefficient

The precipitation coefficient was assessed during the second protein recovery stage at various inlet flow rates (5, 10, 15 L/h). Table 6 presents the results of testing each flow rate for 1.5 hours. The data shows that the amount of precipitate (Precipitate wet mass) increased with higher inlet flow rates, as more LLP was pumped into the decanter during the run time. Upon increasing the inlet flow rate, it was observed that the precipitation coefficient at pH 3.5 increased as it reached its highest point at 10 L/h, but then decreased when the inlet flow rate was increased to 15 L/h. It is worth noting that any protein not precipitated remained present in the LPUP. The reason for this is that not all proteins were affected by acid precipitation, as it depends on their individual isoelectric point (pI) values which are specific to each protein. The goal of this study was to extract the maximum amount of protein from the RPC, instead of focusing on separating specific proteins, which is commonly known as protein fractionation.

It is important to note that Table 6 displays the results without the additional surface scraping procedure, whereas Table 7 shows the results when performing such a procedure. The latter resulted in significantly higher quantities of precipitate and substantially higher precipitation coefficients. The values obtained are more aligned with the research conducted by Ahlström et al. in 2022. According to their findings, the precipitation coefficient was at its highest level, reaching 65%, when utilizing the alkaline extraction method and a pH 3.5 during the precipitation step [19].

Table 6. Precipitation coefficient without scrapping the equipment's surfaces off. Different letters in each row indicate a significant difference ($p \leq 0.05$).

Flow rate (L/h)	**5**	**10**	**15**
Pumped LLP wet mass (g)	6200	11000	21000
Protein content of LLP (db %)	33.4 ± 0.2^{a}	33.4 ± 0.2^{a}	33.4 ± 0.2^{a}
Mass of protein LLP (g)	140	260	470
Precipitate wet mass (g)	140	530	980
Protein content of precipitate (db %)	51.5 ± 0.4^{a}	51.4 ± 0.3^{a}	49.3 ± 0.2^{a}
Mass of protein precipitate (g)	19	73	120
Precipitation coefficient (%)	13	28	26

Up until this point, the methodology used in the study did not consider this particular factor. However, due to the difficult handling of the highly viscous precipitate obtained during this stage, a substantial amount of solid was discovered to be stuck at the solids' discharge. Therefore, it was regarded as valuable to evaluate its effect on the precipitation coefficient and protein recovery yield.

Table 7. Precipitation coefficient scrapping the equipment's surfaces off. Different letters in each row indicate a significant difference ($p \leq 0.05$).

Flow rate (L/h)	**10**	**15**
Pumped LLP wet mass (g)	11000	21000
Protein content of LLP (db %)	33.4 ± 0.2^{a}	33.4 ± 0.2^{a}
Mass of protein LLP (g)	260	470
Precipitate wet mass (g)	910	1400
Protein content of precipitate (db %)	51.3 ± 0.3^{a}	49.3 ± 0.2^{a}
Mass of protein precipitate (g)	120	180
Precipitation coefficient (%)	48	38

It is possible that the decrease in precipitation coefficient is a result of the varying settling times of the LLP buckets that were collected previously and had their pH adjusted to 3.5. This means that each bucket settled at different times before being utilized in the experiments. Although the trials were intended to be continuous, the availability of the decanter centrifuge was limited during the final stage of the study due to increased demand. A new batch of RPC was used for the second protein recovery step, which may have affected the protein content compared to the previous batch.

3.3.3. Protein recovery yield

The protein recovery yield was calculated for both procedures with and without scraping the equipment surfaces. Based on the data presented in Table 8, it is evident that the overall yield varied at different inlet

flow rates. Specifically, it reached its highest point at 10 L/h, but then decreased when the inlet flow rate was increased to 15 L/h. In addition, after comparing the two procedures, it was found that yields were notably higher when scraping off any material build-up on the equipment's surface. This indicates that a considerable amount of material was recovered from the walls of the equipment. Although this extra step only occurred during the second step of the protein recovery methodology, specifically when the inlet flow rates were 10 and 15 L/h, it provided a useful estimate of the overall protein recovery yield.
Based on the results of the performance evaluation and the extraction and precipitation coefficients and protein recovery yield, it was determined that a flow rate of 10 L/h alongside scrapping the surface of the equipment would be the best option. This choice will significantly enhance the overall capacity of the process without drastically affecting important aspects such as the dry matter and protein content of the precipitate. To demonstrate, the highest values for protein precipitation coefficient and protein recovery yield were observed at a flow rate of 10 L/h, with percentages of 48% and 29%, respectively.

Table 8. RPC protein recovery yield with and without scrapping the equipment's surfaces off.

Flow rate (L/h)	Yield without scrapping off (%)	Yield scrapping off (%)
5	10	N/A
10	17	29
15	16	23

Since the protein recovery yield is directly affected by the precipitation process (See equation 3) performed during the second protein recovery step, the same behaviour was observed. It was observed that when the product was pumped into the decanter at a rate of 10 L/h, there was a slightly higher protein recovery yield compared to when it was pumped at 15 L/h. In a previous study assessing the effect of upstream mechanical processing of RPC on the protein recovery yield, conducted by Baker et al. in 2022, a pilot scale two-step protein recovery process was used to achieve an average yield of 14%. The process involved soaking of RPC for 24 hours, followed by homogenization using a colloidal mill. Thereafter, the pH was adjusted to 7, and the mixture was finally processed through a decanter centrifuge [24]. The protein recovery yield achieved in the current study cannot be compared directly to that of the study conducted by Baker et al. This is due to the differences in methodology between the two studies. However, the latter study does suggest that optimising the salt extraction method is a promising approach. In 2022, Helstad et al. conducted another study on protein recovery from hempseed press cake on a pilot scale. The process involved using a constant alkali extraction pH of 10.5 with continuous stirring for 4 hours, followed by isoelectric precipitation at pH 5.5. The findings revealed a protein recovery yield of 57% [25].

It is important to note that in order to obtain a more precise calculation of the protein recovery yield, the actual amount of LLP pumped and the resulting precipitate was projected by assuming that the total amount of LLP obtained during the initial protein recovery step had been pumped. This method ensured a more accurate representation of the protein recovery yield. Due to time constraints, each inlet flow rate (5, 10 and 15 L/h) was only tested for 1.5 hours and thus it was not possible to determine the actual amount of precipitate that would be obtained.

4. Conclusion

When it comes to utilizing a decanter centrifuge for separation processes and optimizing the protein recovery of RPC, a linear behaviour cannot be assumed. Relying solely on findings from laboratory-scale setups may not accurately estimate overall performance. It is, therefore, crucial to consider this limitation to achieve optimal results in protein recovery. It is also crucial to note that the set of processing parameters for each application varies based on the desired outcome and the intrinsic properties of the fluid being used. With this in mind, to improve process capacity without compromising dry matter and protein content of the LPP during the first protein recovery step, it was decided to employ the following set of processing parameters: 60 L/h inlet feed rate, 2000xg acceleration, 50 rpm differential speed, and 68 mm WD. These parameters resulted in 6.90 ± 0.0% and 33.4 ± 0.2% dry matter and protein content, respectively. At this flow rate, the combination of dry matter and protein content values of LLP was found to be the most optimal compared to those obtained at 40L/h and 80L/h. As the aim was to extract proteins using a gentler approach, pH 7 was still deemed appropriate and, in combination with the addition of NaCl, yielded a 61% extraction coefficient.

During the initial protein recovery step, the inlet flow rate, differential speed, and WD were found to significantly impact the equipment's operability. If the differential speed was low (10 rpm) and the inlet flow rates were high (40 L/h, 60 L/h, and 80 L/h), there was an unexpected increase of solids in the bowl and excessive torque in the screw conveyor, triggering the automatic decanter shutdown feature for safety. However, this issue was avoided by setting the differential speed to 50 rpm. Therefore, operating the equipment at maximum capacity may not result in better separation performance or equipment operability.

Moreover, the precipitation coefficient was assessed during the second protein recovery stage at various inlet flow rates (5, 10, 15 L/h) and at a fixed acceleration value of 4000xg, differential speed of 6 rpm and WD inner diameter of 56mm. Up until this point, the study's approach did not consider the losses of the product caused by fouling. However, the handling of the highly viscous precipitate during this phase proved to be challenging, resulting in a significant amount of solid material getting stuck at the solids' discharge. As a result, it was regarded crucial to assess its impact on both the precipitation coefficient and the protein recovery yield. When the inlet flow rate was increased, there was an observable increase in the precipitation coefficient at pH 3.5. By pumping the LLP at 10 L/h without scraping the surface, the precipitation coefficient rose to 28 %. However, performing the additional procedure resulted in an even higher precipitation coefficient of 48%. At a flow rate of 15 L/h, these values decreased to 26 % without scraping and 38 % with the additional procedure.

Based on the results of the study, it was determined that scraping the equipment surfaces significantly improves protein recovery yield. Specifically, the trials showed that at an inlet flow rate of 10 L/h, the yield increased from 17 % without scraping to 29 % when the surface was scraped. Similarly, at 15L/h, the yield increased from 16% to 23% when the surface was scraped. In cases where the processing is disrupted, as was the case in this study, surface scraping is crucial to improve process capability. However, if the equipment runs continuously, the results may differ due to the high volume of production over extended periods. In these situations, the accumulation of material is likely to become negligible compared to the substantial amount generated. Lastly, through careful evaluation, it was concluded that a flow rate of 10 L/h would be the most suitable option. This decision will greatly improve the process's overall ability without negatively impacting crucial factors like the dry matter and protein content of the precipitate.

5. Future work

To enhance the process, it's essential to prioritize optimizing the upstream of process first. This will make the proteins more accessible and easier to extract using the salt extraction method. It will also make it more convenient to handle the RPC dispersion being pumped at the beginning of the process and the precipitate later on in the downstream. This is because it was observed that the spent solid fraction and precipitate got stuck on the walls of the equipment due to its highly viscous behaviour. By taking this approach, product loss can be minimized, and protein recovery yield can be improved. For instance, adding a defatting procedure on the RPC before proceeding with the extraction could be investigated. Furthermore, as prior research has indicated, both pH and temperature are important factors in the protein extraction process from RPC. While this project only focused on pH, it is crucial to also analyse the impact of temperature on the process's protein recovery abilities, particularly during the initial protein extraction phase. This would allow for a more comprehensive understanding of the process.

Additionally, it is advisable to assess the second protein recovery process at a reduced differential speed to prolong the duration that solids remain in the decanter bowl, specifically a value within the range of 1 and 5 rpm. This could lead to improved separation efficiency and higher protein content in the precipitate. Unfortunately, the project was unable to investigate the inlet flow rate further due to time constraints. Nonetheless, it is crucial to determine the actual capacity of the equipment for the second protein recovery step, specifically isoelectric precipitation. It is important to consider the equipment's ability to produce precipitate without causing any negative impact on its operability and the resulting product loss. The focus should always be on achieving the best possible outcome in terms of dry matter and protein content of the precipitate. During equipment design evaluations, it was discovered that although the equipment has two scrappers at the discharge of solids, the material still adheres to the walls. To ensure efficient protein recovery and avoid product loss, it is recommended to enhance the scrapping mechanism for viscous solid fractions.

References

1. Ahlström C, Thuvander J, Rayner M, Mayer Labba I-C, Sandberg A-S, Östbring K. Pilot-Scale Protein Recovery from Cold-Pressed Rapeseed Press Cake: Influence of Solids Recirculation. Processes. 2022;10(3):557.
2. Ma KK, Greis M, Lu J, Nolden AA, McClements DJ, Kinchla AJ. Functional Performance of Plant Proteins. Foods. 2022;11(4):594.
3. Wanasundara JPD, Tan S, Alashi AM, Pudel F, Blanchard C. Chapter 18 - Proteins From Canola/Rapeseed: Current Status. In: Nadathur SR, Wanasundara JPD, Scanlin L, editors. Sustainable Protein Sources. San Diego: Academic Press; 2017. p. 285-304.
4. Commission E. Oilseeds and Protein Crops market situation. European Union; 2023.
5. Tan S, Mailer R, Blanchard C. Canola Proteins for Human Consumption: Extraction, Profile, and Functional Properties. Journal of food science. 2011;76:R16-28.
6. Gunnarshögs. Manufacturing: Gunnarshögs Jordbruks AB; 2023. Available from: https://www.gunnarshog.se/tillverkning/. (Accessed on 9 May 2023).
7. Östbring K, Malmqvist E, Nilsson K, Rosenlind I, Rayner M. The Effects of Oil Extraction Methods on Recovery Yield and Emulsifying Properties of Proteins from Rapeseed Meal and Press Cake. Foods. 2020;9(1):19.
8. Jia W, Rodriguez-Alonso E, Bianeis M, Keppler JK, van der Goot AJ. Assessing functional properties of rapeseed protein concentrate versus isolate for food applications. Innovative Food Science & Emerging Technologies. 2021;68:102636.
9. Zhang J. Protein-protein interactions in salt solutions. Protein-protein interactions-computational and experimental tools. 2012;6:359-376.
10. Biocompare. Benchtop Centrifuges: Biocompare; 2023. Available from: https://www.biocompare.com/Lab-Equipment/7667-Benchtop-Centrifuges/. (Accessed on 29 April 2023).
11. Pak T. Dairy Processing Handbook. Lund, Sweden: Tetra Pak Processing Systems AB; 2015. p. 128-130.
12. Flottweg. The Flottweg decanter: Flottweg SE; 2023. Available from: https://www.flottweg.com/product-lines/decanter/. (Accessed on 28 April 2023).
13. Laval A. Optimizing fish processing with decanters: Alfa Laval Corporate AB; 2015. Available from: https://www.alfalaval.com/media/stories/food-processing/optimizing-fish-processing-with-decanters/. (Accessed on 2 May 2023).
14. Flottweg. The Features and Success Factors of Our Decanter Centrifuges: Flottweg SE; 2023. Available from: https://www.flottweg.com/engineering/decanter-features/. (Accessed on 29 April 2023).
15. Flottweg. Centrifuge Speed and Acceleration: Flottweg SE; 2023. Available from: https://www.flottweg.com/wiki/separation-technology/centrifuge-speed-and-acceleration/.(Accessed on 29 April 2023).
16. Laval A. Decanter Operating Principle: AB, Alfa Laval Corporate; 2023. Available from: https://www.alfalaval.com/globalassets/documents/products/separation/centrifugal-separators/decanters/alfa-laval-decanter-centrifuge-operating-principles.pdf. (Accessed on 3 May 2023).
17. Ekwall SN. Optimization of the second separation step for precipitation of proteins from rapeseed press cake. Lund, Sweden: Lund University; 2021.
18. Lemitec. Lemitec MD 80 decanter centrifuge: Lemitec GMBH; 2023. Available from: https://www.lemitec.com/en/products/md80.php. (Accessed on 18 April 2023).
19. Ahlström C, Thuvander J, Rayner M, Matos M, Gutiérrez G, Östbring K. The Effect of Precipitation pH on Protein Recovery Yield and Emulsifying Properties in the Extraction of Protein from Cold-Pressed Rapeseed Press Cake. Molecules. 2022;27(9):2957.

20. Laval A. Laboratory tests: Alfa Laval Corporate AB; 2023. Available from: https://explore.alfalaval.com/lab-test. (Accessed on 3 May 2023).
21. Pelegrine DHG, Gasparetto CA. Whey proteins solubility as function of temperature and pH. LWT - Food Science and Technology. 2005;38(1):77-80.
22. Rodrigues I, Carvalho G, Rocha J. Increase of protein extraction yield from rapeseed meal through a pretreatment with phytase. Journal of the Science of Food and Agriculture. 2017;97(8):2641-2646.
23. Fetzer A, Herfellner T, Stäbler A, Menner M, Eisner P. Influence of process conditions during aqueous protein extraction upon yield from pre-pressed and cold-pressed rapeseed press cake. Industrial Crops and Products. 2018;112:236-246.
24. Baker PW, Višnjevec AM, Krienke D, Preskett D, Schwarzkopf M, Charlton A. Pilot scale extraction of protein from cold and hot-pressed rapeseed cake: Preliminary studies on the effect of upstream mechanical processing. Food and Bioproducts Processing. 2022;133:132-139.
25. Helstad A, Forsén E, Ahlström C, Mayer Labba I-C, Sandberg A-S, Rayner M, et al. Protein extraction from cold-pressed hempseed press cake: From laboratory to pilot scale. Journal of Food Science. 2022;87(1):312-325.

Appendix

Table A. LLP sample nomenclature established for different combinations of processing parameters that were assessed during the first protein recovery step.

Sample ID	Inlet flow rate (L/h)	Acceleration (g)	Differential speed (rpm)	WD inner diameter (mm)
Benchmark	20	2000	10	56
40115	40	1000	10	56
40116	40	1000	10	68
40155	40	1000	50	56
40156	40	1000	50	68
60115	60	1000	10	56
60116	60	1000	10	68
60155	60	1000	50	56
60156	60	1000	50	68
80115	80	1000	10	56
80116	80	1000	10	68
80155	80	1000	50	56
80156	80	1000	50	68
40215	40	2000	10	56
40216	40	2000	10	68
40255	40	2000	50	56
40256	40	2000	50	68
60215	60	2000	10	56
60216	60	2000	10	68
60255	60	2000	50	56
60256	60	2000	50	68
80215	80	2000	10	56
80216	80	2000	10	68
80255	80	2000	50	56
80256	80	2000	50	68
40315	40	3000	10	56
40316	40	3000	10	68
40355	40	3000	50	56
40356	40	3000	50	68
60315	60	3000	10	56
60316	60	3000	10	68
60355	60	3000	50	56

60356	60	3000	50	68
80315	80	3000	10	56
80316	80	3000	10	68
80355	80	3000	50	56
80356	80	3000	50	68
40415	40	4000	10	56
40416	40	4000	10	68
40455	40	4000	50	56
40456	40	4000	50	68
60415	60	4000	10	56
60416	60	4000	10	68
60455	60	4000	50	56
60456	60	4000	50	68
80415	80	4000	10	56
80416	80	4000	10	68
80455	80	4000	50	56
80456	80	4000	50	68

Table B. Dry matter and protein content of LLP at different set of processing parameters employing an inner diameter WD of 56 mm.

Sample ID	Dry matter (%)	Average dry matter (%)	Protein content DB (%)	Average protein content DB (%)
40115	5.81	5.81 ± 0.0	32.7	32.8 ± 0.1
	5.81		32.9	
40215	5.63	5.62 ± 0.0	33.0	32.9 ± 0.1
	5.61		32.9	
40315	5.65	5.65 ± 0.0	32.5	32.6 ± 0.1
	5.65		32.6	
40415	5.41	5.42 ± 0.0	33.5	33.5 ± 0.0
	5.43		33.5	
40155	6.07	6.06 ± 0.0	32.7	32.6 ± 0.1
	6.05		32.5	
40255	5.81	5.79 ± 0.0	32.6	32.7 ± 0.1
	5.77		32.7	
40355	5.84	5.85 ± 0.0	32.1	32.2 ± 0.1
	5.85		32.2	
40455	5.68	5.68 ± 0.0	32.7	32.6 ± 0.1
	5.68		32.5	
60115	6.22	6.22 ± 0.0	33.2	33.0 ± 0.3
	6.23		32.8	

60215	5.94	5.94 ± 0.0	32.5	32.6 ± 0.2
	5.93		32.7	
60315	5.98	5.97 ± 0.0	32.3	32.2 ± 0.2
	5.96		32.1	
60415	N/A	N/A	N/A	N/A
	N/A		N/A	
60155	6.33	6.33 ± 0.0	32.0	32.1 ± 0.1
	6.32		32.1	
60255	5.99	5.98 ± 0.0	32.8	33.0 ± 0.3
	5.96		33.2	
60355	5.95	5.94 ± 0.0	33.6	34.1 ± 0.7
	5.93		34.6	
60455	5.91	5.91 ± 0.0	33.6	33.1 ± 0.7
	5.91		32.6	
80115	6.91	6.89 ± 0.0	33.7	34.3 ± 0.8
	6.86		34.8	
80215	N/A	N/A	N/A	N/A
	N/A		N/A	
80315	N/A	N/A	N/A	N/A
	N/A		N/A	
80415	N/A	N/A	N/A	N/A
	N/A		N/A	
80155	6.97	6.95 ± 0.0	34.5	34.5 ± 0.0
	6.93		34.5	
80255	6.28	6.29 ± 0.0	34.0	33.7 ± 0.5
	6.30		33.4	
80355	6.23	6.20 ± 0.0	33.8	33.9 ± 0.1
	6.17		34.0	
80455	5.98	5.98 ± 0.0	33.2	33.3 ± 0.2
	5.99		33.5	

Table C. Dry matter and protein content of LLP at different set of processing parameters employing an inner diameter WD of 68 mm.

Sample ID	Dry matter (%)	Average dry matter (%)	Protein content DB (%)	Average protein content DB (%)
40116	6.63	6.61±0.0	32.7	32.7 ± 0.0
	6.60		32.7	
40216	N/A	N/A	N/A	N/A
	N/A		N/A	
40316	N/A	N/A	N/A	N/A
	N/A		N/A	

40416	N/A	N/A	N/A	N/A
	N/A		N/A	
40156	7.20	7.16 ± 0.1	32.0	31.9 ± 0.1
	7.12		31.8	
40256	6.39	6.42 ± 0.0	34.0	34.1 ± 0.0
	6.44		34.1	
40356	6.21	6.20 ± 0.0	33.0	33.0 ± 0.0
	6.19		33.0	
40456	6.16	6.15 ± 0.0	32.6	32.5 ± 0.1
	6.14		32.4	
60116	7.33	7.31 ± 0.0	32.0	32.0 ± 0.1
	7.28		31.9	
60216	N/A	N/A	N/A	N/A
	N/A		N/A	
60316	N/A	N/A	N/A	N/A
	N/A		N/A	
60416	N/A	N/A	N/A	N/A
	N/A		N/A	
60156	7.37	7.35 ± 0.0	32.0	32.1 ± 0.0
	7.32		32.1	
60256	6.93	6.90 ± 0.1	33.5	33.4 ± 0.2
	6.86		33.2	
60356	6.60	6.61 ± 0.0	33.2	33.0 ± 0.2
	6.61		32.9	
60456	6.24	6.24 ± 0.0	33.3	33.2 ± 0.1
	6.24		33.2	
80116	7.92	7.90 ± 0.0	31.0	30.8 ± 0.3
	7.89		30.6	
80216	N/A	N/A	N/A	N/A
	N/A		N/A	
80316	N/A	N/A	N/A	N/A
	N/A		N/A	
80416	N/A	N/A	N/A	N/A
	N/A		N/A	
80156	7.69	7.71 ± 0.0	31.3	31.3 ± 0.0
	7.74		31.3	
80256	7.10	7.02 ± 0.1	32.7	32.9 ± 0.3
	6.94		33.1	
80356	6.66	6.63 ± 0.0	33.7	33.6 ± 0.11
	6.60		33.5	
80456	6.54	6.55 ± 0.0	34.1	34.0 ± 0.1

	6.56		34.0	