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Process design for sustainable extraction of rapeseed protein mixtures

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ABSTRACT

Natural sustainable emulsifiers from plants are getting more attention of the food industry as alternative for animal-based emulsifiers. A potential plant protein source is rapeseed. During conventional rapeseed oil extraction, a protein-rich meal side stream is produced. However, protein extraction from this meal by-product is difficult, because the harsh conditions applied for oil extraction highly influence the nativity and solubility of the proteins present in the meal. The purpose of this study is to investigate the feasibility of a novel aqueous extraction process for protein extraction from intact rapeseed, which uses mild extraction conditions. In this process a protein concentrate is produced, as well as two side-products: oil body cream and pellet.

In this study, the novel aqueous extraction process is compared with state-of-the-art competitive processes of the University of Toronto and Burcon NutraScience Corporation©, which extract protein from rapeseed meal. Based on literature, patent publications and laboratory analysis data, process models were constructed for each process to compare key process performance parameters on product yield and quality, energy consumption and water consumption. Also, the environmental impact in terms of waste stream generation, wastewater production and processing related CO_2 emission was evaluated.

The results of this study show that the aqueous extraction process is feasible in terms of protein recovery. The protein recovery of the designed process is 68% of total rapeseed protein, when also the side-products are valorised. Because the products contain native proteins and functional emulsifiers due to the mild extraction process, a full valorization of all products is most likely feasible. The protein yield of the protein concentrate is 39%. This yield is higher than the protein yields of the protein isolates produced in the benchmark processes (20% Burcon and 32% Toronto). Energy consumption of the designed process (1.74-2.04 MJ per kilogram rapeseed processed) is slightly lower compared to the benchmark process of Burcon (2.24-2.52 MJ per kilogram rapeseed processed).

The cost price range of the protein concentrate produced in the designed process is between 9.55-18.59 euro per kilogram, when side-products are considered to have no market value. This cost price is high, compared to the benchmark processes of Burcon and Toronto, which show cost prices in the range of 3.33-4.73 and 7.61-12.99 euro per kilogram protein isolate, respectively. However, the total cost price of the products in the aqueous extraction process is much lower when also the side-products are valorised (0.47-0.91 euro per kilogram). Based on the weighted average market value of all products (0.75-11.33 euro per kilogram), the aqueous extraction process becomes economically feasible if high product quality and value is achieved.

Further scaling up to a proof of practice is necessary to optimize efficiencies and study functional properties in more detail.

CONTENTS

1 INTRODUCTION

Many food products consist of oil-in water emulsions, in which small oil droplets are dispersed in an aqueous environment. These emulsions are thermodynamically not stable and emulsifying agents are needed for stabilization of the oil to water interfaces [1]. Currently applied protein-based emulsifiers are predominantly of animal origin. For example, casein and whey proteins derived from bovine milk and proteins derived from egg [1], [2]. For the production of one kilogram of animal protein, about eight kilograms of plant protein are required [3]. Besides, production of animal-based foods has a large environmental impact and is not sustainable [4]. Therefore, plant protein sources are being exploited as an alternative to replace animal-based emulsifiers. A potential plant protein source is rapeseed.

1.1 RAPESEED STRUCTURE AND COMPOSITION

SEED STRUCTURE

A mature rapeseed seed consists of two seed components: the seed coat (i.e. hull) and the embryo (i.e. kernel). The seed coat consists of the epidermis, palisade and aleuronic layer and the embryo is made up of the radicle and cotyledons (figure 1A) [5].

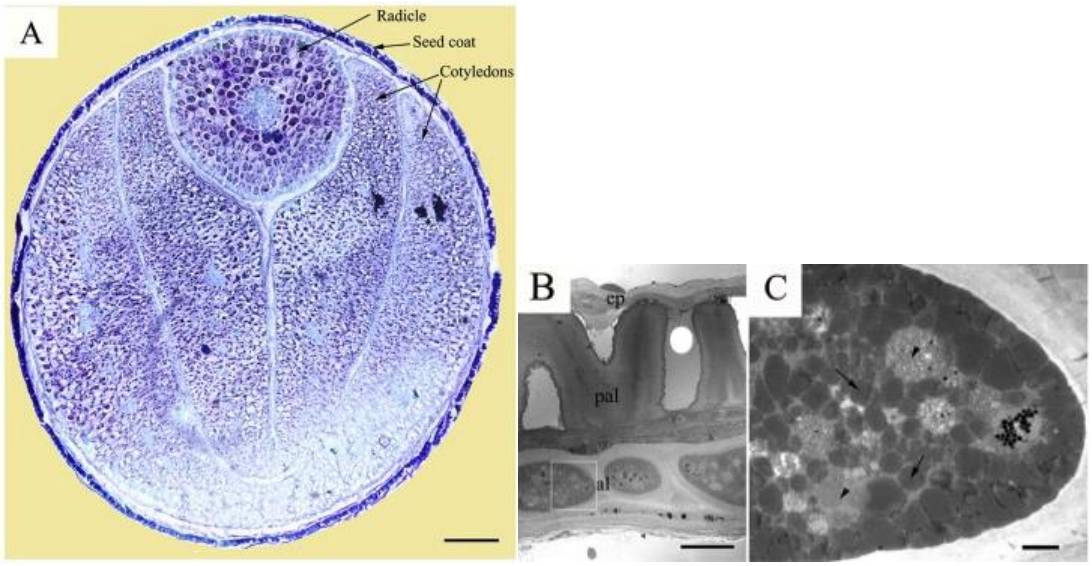

FIGURE 1: A) CROSSSECTION OF A MATURE *B. NAPUS* SEED. B) PICTURE OF THE SEED COAT SHOWING THE EPIDERMIS, PALISADE AND ALEURONIC LAYER. C) STRUCTURE OF THE ALEURONIC CELL ENCIRCLED IN B), SHOWING PROTEIN BODIES WITH ARROWHEADS AND OILBODIES WITH ARROWS. *ADAPTED FROM: HU, ZHI-YONG, ET AL. "SEED STRUCTURE CHARACTERISTICS TO FORM ULTRAHIGH OIL CONTENT IN RAPESEED." PLOS ONE 8.4 (2013): E62099.*

The seed embryo is the main storage place for proteins and oil. Proteins and oil are stored in the in the form of protein and oil bodies. Oil and protein are stored to save energy and nitrogen for utilization during germination. Protein and oil bodies are also present in the aleuronic layer of the seed hull (figure 1B/C) [5].

COMPOSITION

B. rapa and *B. napus* seeds are reported to have a similar composition [6]. The seeds consist mostly of oil and protein, followed by carbohydrates, lignin and ash (table 1).

TABLE 1: APPROXIMATE COMPOSITION OF RAPESEED ON MOISTURE FREE BASIS (WT.% DM) [6]

Oil	Protein	Carbohydrates	Lignin	Ash
45%	22%	13%	5%	4%

1.2 PRODUCTION VOLUME

The high amount of oil makes rapeseed the largest produced oil crop around the globe after soybean. Rapeseed is therefore primarily processed for oil extraction. In 2018, 73.95 million metric tons of rapeseed is cultivated worldwide, which is 13% of total oilseed cultivation [7]. Most rapeseed is cultivated in Europe, when in fact Canada, China and India are the largest rapeseed production countries (table 2).

TABLE 2: RAPESEED PRODUCTION IN THOUSAND METRIC TONS

Country/region	2017/2018
European Union	22,100
Canada	21,500
China	14,400
India	5,700
Other	10,249

Source: Foreign Agricultural Service. USDA. 2018. Oilseed: World Markets and Trade.

The amount of oilseeds that is grown has increased over the past years [7]. This is because vegetable oil is more and more used as source for production of renewable biodiesel. Rapeseed oil is also applied in food products, partially due to its recognized beneficial health effects.

1.3 CONVENTIONAL OIL EXTRACTION PROCESS

As mentioned before, the oil is stored in oil bodies. Oil bodies are intracellular spherical organelles surrounded by a monolayer of phospholipids embedded with some proteins, mostly oleosin and some minor proteins called caleosin and steroleosin [8]–[10]. These proteins establish the integrity of oil bodies in rapeseed cells and ensure that the oil stays naturally emulsified in the seed [11].

The oil extraction process is an established industrial process, which focuses on obtaining high oil extraction efficiencies, disrupting the natural structure of oil bodies. The process is schematically shown in figure 2.

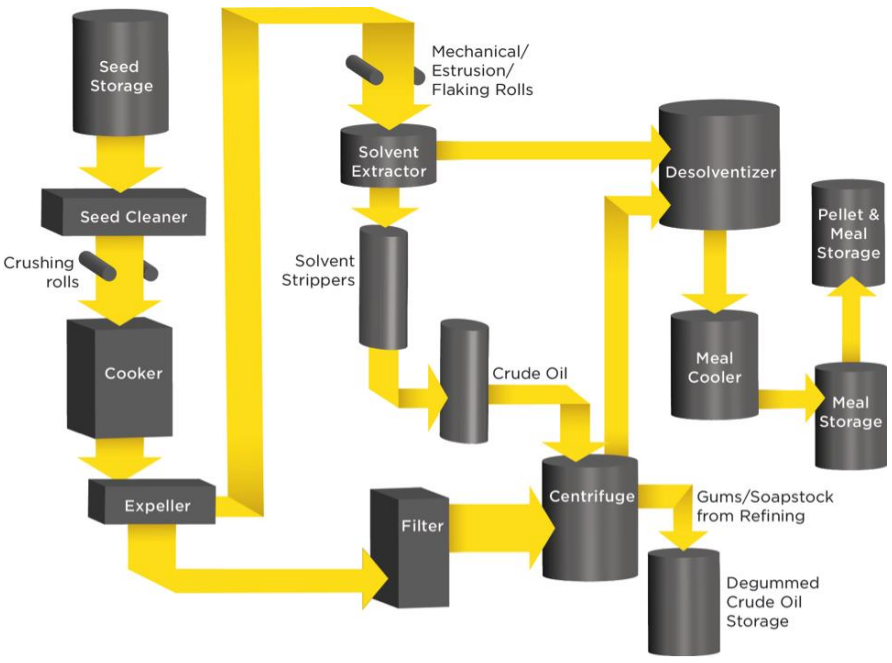

FIGURE 2: COMMERCIAL RAPESEED OIL EXTRACTION PROCESS. *ADAPTED FROM: CANOLA COUNCIL OF CANADA (2013). FROM THE FIELD TO THE FEED BUNK: CANOLA MEAL'S JOURNEY* [12].

The seeds are first cleaned after which they are flaked by crushing rolls to rupture the seed coat. The cracked seeds are then cooked using steam at 80-105 °C to further open up the cells. In this step the enzyme myrosinase, which can convert glucosinolates into toxic compounds, is inactivated. The next step is screw pressing or expeller pressing of the flakes to mechanically extract the oil [13]. The resulting cake still contains some residual oil (15-22%), which is subsequently recovered by hexane extraction [14]. Some hexane will remain in the meal after extraction. The remaining solvent is removed by a desolventizer-toaster, in which steam heating of the meal to 130 °C will evaporate most of the hexane remain [14]. This protein-rich rapeseed meal side-product is currently mainly used as fodder [2].

The crude oil produced in this process can be further refined to meet the quality demands for application in the food industry. These refining steps often include bleaching and deoderization steps. The high temperatures applied in the oil extraction process for evaporation of residual solvent negatively influence meal protein quality and solubility [2].

According to a survey which included ten rapeseed oil extraction plants, the electricity requirement for production of crude oil is on average 49 Wh electricity per kilogram of rapeseed processed, whereas the heat requirement is around 1000 kJ natural gas per kilogram of rapeseed processed [13].

A well-operated rapeseed oil extraction plant typically requires three litres of hexane per ton of rapeseed processed, which corresponds to 0.002 kilogram of hexane per kilogram or rapeseed processed, because most of the hexane used during extraction is subsequently recovered [15].

1.4 POTENTIAL OF RAPESEED PROTEINS

As explained, rapeseed proteins can potentially be applied in the food industry to replace animal-based emulsifiers, or to be used as gelling or foaming agents [2]. The most abundant proteins in rapeseed are the globular seed storage proteins cruciferin and napin. Cruciferin and napin are stored in protein bodies and account for 60% and 20% of total rapeseed protein, respectively [16]. Cruciferin is a salt soluble 11S globulin with molecular weight of 300-350 kDa and an isoelectric point between pH 4 and pH 7 [17], [18]. Napin is a water-soluble 2S albumin with a weight of 12-16 kDa. Napin is a basic protein and has an isoelectric point of pH 11 [17]. The two proteins have different sizes, molecular structures, amino acid composition and physicochemical properties [17].

The potential of rapeseed protein isolates in terms of functional ingredients in food products depends highly on the protein quality and composition of the final protein isolate. The levels of cruciferin and napin in the protein product can influence its techno-functional properties [2]. In addition, processing conditions applied in oil and protein extraction can also have an effect on the techno-functional properties of the final protein isolate [2].

1.5 RAPESEED PROTEIN EXTRACTION STRATEGIES

A lot of research has been done on process design for extraction of rapeseed proteins. Already since the 1970s, different processes have been developed for protein extraction from rapeseed meal [3]. Protein extraction from the meal by-product is the benchmark process strategy for rapeseed protein extraction (figure 3).

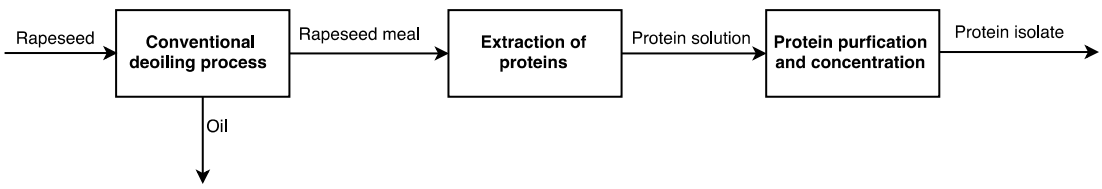

FIGURE 3: BENCHMARK PROCESS STRATEGY OF PROTEIN EXTRACTION FROM RAPESEED MEAL

In this strategy the proteins in the meal are extracted, often by applying alkaline or saline conditions to improve the solubility of the proteins [3]. The resulting protein solution is then purified from non-protein compounds, such as salt and carbohydrates or phenolic compounds that are co-extracted.

Protein extraction from rapeseed meal is challenging and requires harsh conditions (high temperature and alkaline conditions). These harsh conditions might cause denaturation and decreased solubility of the proteins, generally resulting in low extraction yields from the meal [2]. In addition, secondary metabolites, such as glucosinolates and phenolic compounds, can interact with the proteins and result in products with undesired functional properties and dark color [17].

The resulting relatively low protein functionality and yield in combination with the high processing costs necessary for purifying the protein solution, made most protein extraction processes from rapeseed meal not yet economically feasible. In order to allow the extraction of high quality proteins from the meal at reasonable cost, the rapeseed oil extraction process should be adapted. This can be realized by reducing the high temperatures applied in the conventional oil extraction process, for example by cold pressing or low temperature desolventizing [2]. However, this would result in lower oil extraction efficiencies. Because oil is the main focus product of rapeseed oil extraction plants, the focus for a higher meal quality for protein extraction is not common practice. Retrofitting of existing oil extraction plants requires big investments and the market application of high quality meal is still too small to make this economically feasible [19].

Therefore, alternative strategies are being investigated to produce both oil and functional protein products. One such a process strategy is aqueous protein extraction, schematically shown in figure 4.

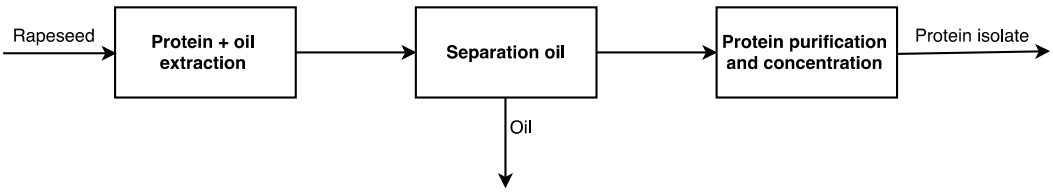

FIGURE 4: PROCESS STRATEGY FOR AQUEOUS PROTEIN AND OIL EXTRACTION FROM RAPESEED

In this developed process strategy, minimal process steps are applied that lead to native protein mixtures with high interfacial activity. In particular, oil and proteins are simultaneously extracted from rapeseed by an aqueous extraction. Subsequently, the extracted oil is recovered in the native form of oil bodies. The resulting protein solution is then purified and concentrated to produce the final protein concentrate. As no heat and organic solvents are required to recover the oil, the proteins are recovered in a more native state.

1.6 PROJECT AIM

Eleni Ntone (Msc), PhD candidate of Wageningen University, has designed a process for aqueous extraction of proteins from rapeseed within the TiFN project “Sustainable ingredients”.

The aim of this project is to compare the designed aqueous extraction process (hereafter called *DAP* process) for simultaneous oil body and protein extraction from rapeseed with other existing state-of-the art processes (hereafter called benchmark processes). The *DAP* process will be compared on defined process performance indicators in terms of product yield and quality, energy consumption, processing related CO_2 emission, water consumption and waste stream generation. The economic feasibility of the *DAP* process for industrial application will also be evaluated and compared with the benchmark processes.

RESEARCH QUESTIONS

- Is the aqueous extraction process favorable in terms of product yield and quality, energy consumption, processing related CO_2 emission, water consumption and waste stream generation?

- Is the aqueous extraction process economically feasible for industrial application?
- Which improvements can be made in the process design?

1.7 APPROACH

The *DAP* process will be compared with existing benchmark processes for rapeseed protein extraction to evaluate its potential. Existing state-of-the art processes designed for protein extraction from rapeseed will be identified by reviewing patents and literature.

A process model of the *DAP* process will be constructed. The model will be used to estimate key process performance parameters such as product yield, energy and water consumption. In addition, the economic feasibility of the process for industrial application will be determined by considering production costs and product revenues. The benchmark processes will also be modeled based on available patent/literature data, to estimate key process performance parameters.

Based on the identified process performance parameters, an overall comparison of the *DAP* process with the benchmark processes is made. The process performance parameters are based on:

- Product yield and quality
- Water consumption
- Energy consumption and processing related CO_2 emission
- Waste streams and side-products
- Cost efficiency and process economics

Based on the benchmark comparison results, the main challenges for the *DAP* process will be identified. Alternative processing steps will be discussed and advised.

2 BACKGROUND INFORMATION

In this section processes that aim for extraction of functional protein isolates from rapeseed, which were selected as benchmark processes, are discussed in detail. Two benchmark processes, which are close to commercial application, were selected for comparison with the *DAP* process. Both selected processes use the benchmark process strategy of protein extraction from rapeseed meal. Furthermore, a detailed process description of the *DAP* process will be given.

2.1 PROCESS DEVELOPED BY THE UNIVERSITY OF TORONTO

2.1.1 PROCESS DESCRIPTION

In the nineties, researchers of the University of Toronto developed a process for protein extraction from defatted rapeseed meal [20]. The process extracts proteins from meal at alkaline conditions (pH 12). After extraction, a part of the proteins is recovered by isoelectric protein precipitation. In isoelectric precipitation, the pH of the protein solution is adjusted to the pH value at which a specific protein has no net electrical charge and thus minimum solubility. These pI values are protein specific and therefore not all rapeseed proteins are precipitated during this step. To recover the remaining soluble protein fraction, membrane technology is applied. During membrane processing the protein solution is first concentrated by ultrafiltration. During ultrafiltration, the protein solution is passed through a semi-permeable membrane that has a specific molecular weight cut-off (MWCO) of 10 kDa. The MWCO allows water and low-molecular weight solutes to pass, while bigger protein molecules are retained in the retentate. In this way, the protein solution is reduced in volume, thus protein concentration is increased. During ultrafiltration, the mass of small impurities is decreased but their concentration is not lowered. To reduce the concentration of impurities, the concentrated protein solution is subsequently purified by diafiltration. Diafiltration is similar to ultrafiltration except that permeated solvent is continuously replaced with fresh solvent. This ensures that the volume of the solution stays constant, while impurities are washed out and lowered in concentration. The final processing step is spray drying of the diafiltered protein solution and the protein precipitate to produce the protein isolates.

The main drawback of this process is the dark color and unpleasant taste of the produced protein isolates, which minimizes their applicability in food products [21]. The bad organoleptic properties are caused mainly by the presence of phenolic compounds, which were co-extracted during alkaline extraction and are bound to rapeseed proteins by a variety of mechanisms [21]. Phenolic compounds can be oxidized to quinones, resulting in undesired dark discoloration of the protein products [21].

Therefore, the process was improved to reduce the levels of phenolic compounds in the protein isolates (figure 5). After extraction, an anti-oxidant (sodium sulfite) is added to the extraction solution to prevent oxidation of co-extracted phenolic compounds. Besides, additional membrane processing, together with a mild heat treatment before precipitation and a polyvinyl pyrrolidone (PVP) treatment after precipitation, was added to the existing process [3]. The mild heat treatment ensures that a portion of the phenolic-protein complexes is broken. This increases the amounts of free phenolic compounds in the solution, which can be removed by membrane technology [22]. Prior to diafiltration, the protein solution is concentrated by ultrafiltration, in order to reduce the amounts of water required during diafiltration [21]. The additional PVP treatment is performed to remove any remaining phenolic compounds in the supernatant after isoelectric precipitation. PVP is an absorbing agent that is able to specifically bind and remove polyphenols [21]. The improved process (hereafter called *TOR* process) produces protein isolates that are up to 80-90% lower in phenolic compounds compared to the original process, while protein yields are not significantly affected by the additional processing steps [21].

2.1.2 PRODUCT CHARACTERISTICS

High pH values applied during extraction, generally result in high protein extraction yields from rapeseed meal [23]. However, the highly alkaline conditions (pH 12) that are applied in this process might lead to denaturation of proteins. Protein denaturation is known to have a negative effect on protein solubility and on associated techno-functional properties of proteins, such as interfacial activity [24]. Besides, highly alkaline conditions can lead to unwanted protein modifications, such as racemization and lysinoalanine formation, which also negatively affect protein functionality [25].

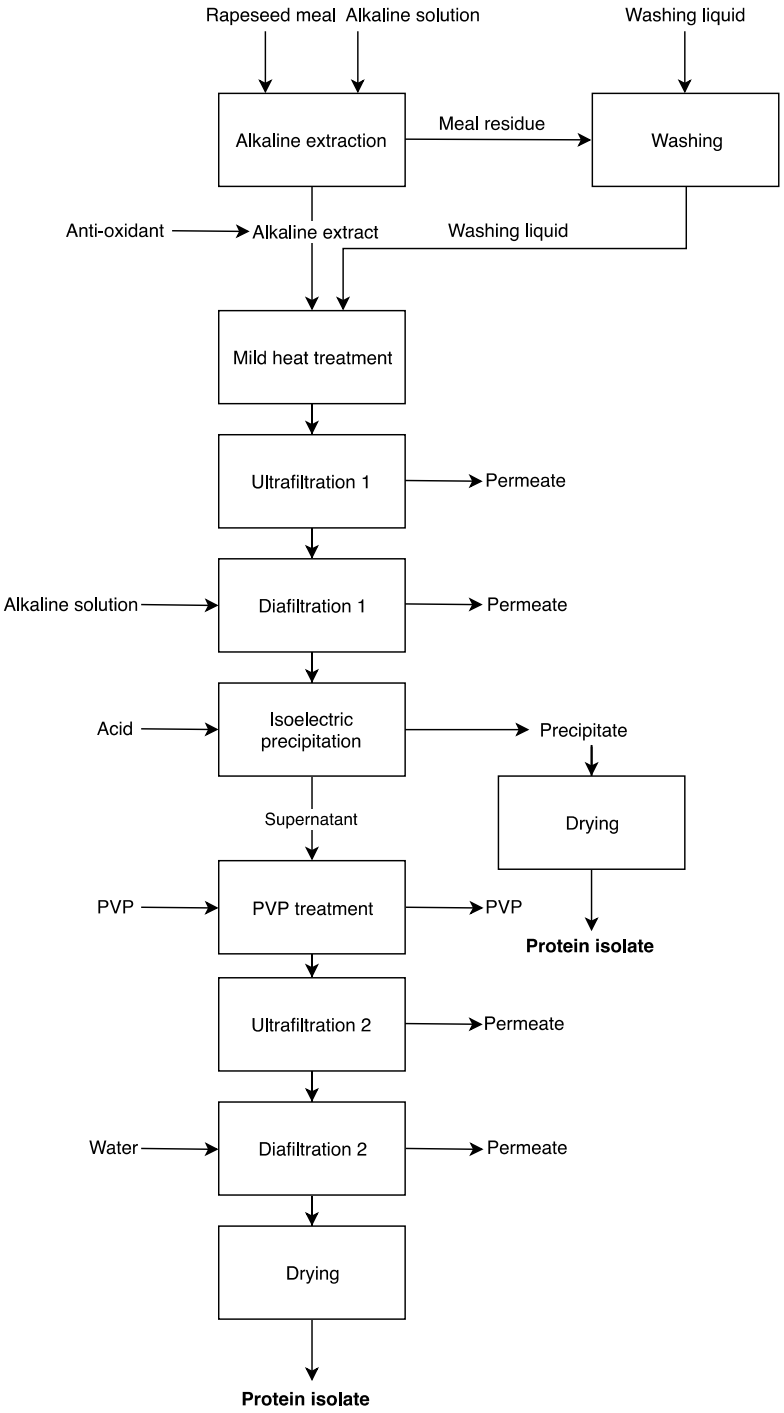

FIGURE 5: PROTEIN EXTRACTION PROCESS DEVELOPED BY THE UNIVERSITY OF TORONTO *ADAPTED FROM: XU, L., DIOSADY, L., 2012. PROCESSING OF CANOLA PROTEINS, IN: THIYAM-HOLLÄNDER, U., ESKIN, M.N.A., MATTHÄUS, B. (EDS.), CANOLA AND RAPESEED. CRC PRESS, BOCA RATON, FL, PP. 59–78.*

2.2 PROCESS DEVELOPED BY BURCON NUTRASCIENCE CORPORATION©

2.2.1 PROCESS DESCRIPTION

Burcon NutraScience Corporation© is a Canadian company specialized in extraction of plant proteins. Burcon NutraScience Corporation© holds many patents on rapeseed protein extraction. The company is currently commercializing an unique process for protein extraction from rapeseed meal (figure 6) [26]. This process will hereafter be referred to as the *BUR* process.

In the *BUR* process, proteins are first extracted from the meal by applying saline extraction conditions. Protein solubilization is enhanced by salt addition, because cruciferin is a salt-soluble globulin (*see Chapter* 1.4) [17]. The residual meal is removed by vacuum filtration after extraction. Residual fine meal particles are subsequently removed from the protein solution by a centrifugation and filtration step. The clarified protein solution is then concentrated to a protein content of over 200 g/L by ultrafiltration [26]. The MWCO of the ultrafiltration membrane is 10 kDa. As a result, most protein molecules are retained, while smaller molecules such as salt, secondary plant metabolites and sugar are permeated. The solution is being enriched in proteins and reduced in volume, but the ionic strength of the solution is retained.

The concentrated protein solution is then diluted with cold water of 4 °C. This dilution step results in a drop in the ionic strength of the solution. The simultaneous drop in temperature and ionic strength causes the formation of a protein micellar mass (PMM). The previous ultrafiltration step was applied to reduce the amounts of cold water needed for a certain drop in ionic strength required for PMM formation.

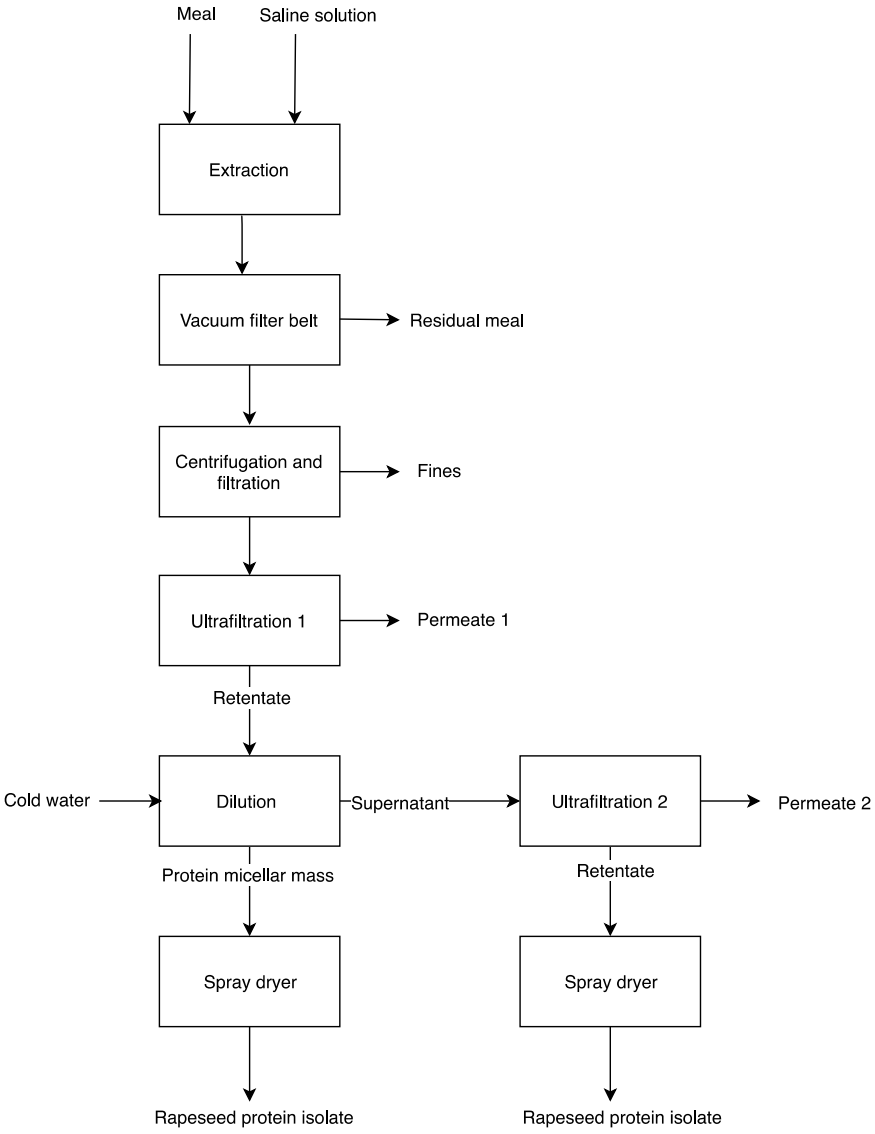

FIGURE 6: PROTEIN EXTRACTION PROCESS DEVELOPED BY BURCON *ADAPTED FROM: BARKER, L.D., MARTENS, R.W. AND MURRAY, E.D. 2010. PRODUCTION OF OIL SEED PROTEIN ISOLATE. US PATENT 0,125,526A1.*

The PMM is allowed to settle, separated and spray dried, resulting in the first protein isolate, which is branded as Puratein©.

A part of the proteins is still present in the supernatant, which is subsequently concentrated by ultrafiltration. Proteins are again retained while smaller impurities are completely permeated. The water added in the dilution step to cause PMM formation, thus also functions to wash out smaller impurities in the subsequent ultrafiltration step. The concentrated protein solution is finally spray dried resulting in the second protein isolate, named Supertein™.

2.2.2 PRODUCT CHARACTERISTICS

The *BUR* process applies saline extraction, which is reported to be beneficial in terms of product functionality compared to alkaline extraction. Saline extraction minimizes protein conformational changes and protein denaturation [23]. A study found that rapeseed protein isolates produced by saline extraction possess higher solubility and interfacial activity compared to isolates produced by alkaline extraction [27]. Another advantage is that fewer undesired phenolic compounds are co-extracted during saline extraction, relative to alkaline extraction [23].

Two functional protein isolates are produced in the *BUR* process. The Puratein© isolate consists largely of the globulin cruciferin, while the Supertein™ isolate contains mostly the albumin napin [28]. The two main seed storage proteins present in rapeseed are thus separated into two fractions and therefore the isolates have different techno-functional properties [2], [28]. The Puratein© isolate can be used in many food applications and specifically has good emulsifying and gelling properties. The Supertein™ product does not have emulsifying capacity but has good nutritional value, high solubility over a wide pH range and good foaming abilities [28].

2.3 DESIGNED AQUEOUS EXTRACTION PROCESS

2.3.1 PROCESS DESCRIPTION

The process flow diagram of the *DAP* process is shown in figure 7.

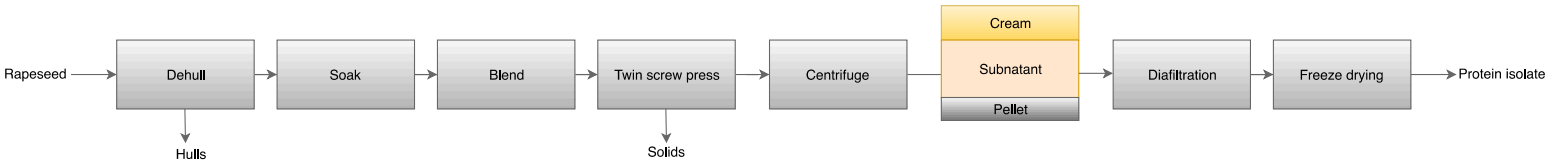

FIGURE 7: DESIGNED AQUEOUS PROTEIN EXTRACTION PROCESS

The first step, dehulling, is for removal of polyphenolic compounds and fibers, which are concentrated in the hulls. The dehulled seeds are subsequently soaked in an alkaline solution of pH 9. The seeds are then mixed to open up the cells and to dissolve both the rapeseed proteins and oil bodies. Next, a screw press operation separates the remaining seed solids, which are mainly composed of cell wall fragments and fibers. The resulting solution is separated by gravity using a centrifuge. The resulting three distinct phases are: the heavier pellet, the subnatant and the lighter oil body cream.

The pellet contains fibers, polysaccharides and some proteins. The oil cream is the lighter top layer that consists of intact oil bodies. The middle phase, called subnatant, is an aqueous solution in which the seed storage proteins cruciferin and napin are dissolved. After the centrifuge separation, the subnatant is further purified by diafiltration, to remove impurities such as sugar and phenolic compounds. The resulting purified solution is freeze-dried to produce the protein concentrate.

2.3.2 PRODUCT CHARACTERISTICS

The downstream processing is focused on maintaining functionality of the products, rather than on full purification. This approach enables the application of simple extraction steps, to obtain interfacial active multicomponent protein systems. Three functional products are produced: the protein isolate, the oil body cream and the pellet. By refining the rapeseed components in three functional fractions, valorization of these components is maximized. The intended use of the produced protein isolate is application as an emulsifier in food emulsions to replace protein emulsifiers of animal origin. The oil body cream side-product can also potentially be applied as emulsifier in food products, as it is naturally emulsified rapeseed oil.

3 MATERIALS AND METHODS

A process model was developed for the *DAP* process and for the *BUR* and *TOR* benchmark processes. The models were used to compare process performance in terms of water consumption, waste stream generation, production yield, product purity, energy/CO_2 footprint and process economics. The models were constructed in a Microsoft® Excel spreadsheet, by setting up mass balances over the individual unit operations. A mass balance is defined as "*a consideration of the input, output and distribution of a substance in a process"* [29]. The mass balances quantify the distribution of the main substances of rapeseed in each unit operation. A major difference between the *DAP* process and the benchmark processes is that the benchmark processes use commercial rapeseed meal as starting material (instead of intact rapeseed). For a proper comparison of the processes, all processes were simulated starting with intact rapeseed. The commercial de-oiling process was therefore included in the models of the *BUR* and *TOR* process.

The main substances of rapeseed were categorized as moisture, oil, protein and 'others' in the models. The category 'others' was introduced as balancing item and corresponds to all other rapeseed components, including the carbohydrates/fibers fraction and the plant secondary metabolites. For each unit operation, mass losses of main components in the waste stream or by-product stream were determined. The model developed for the *DAP* process was validated by provided laboratory data, whereas the models constructed for the competitive processes were validated by key figures identified from literature and patent publications.

3.1 PROCESS PERFORMANCE INDICATORS

Key process performance parameters were calculated in the models in order to compare the processes. Process performance indicators included in the models are listed in table 3 and are elaborated on in detail in this section.

TABLE 3: DEFINED KEY PROCESS PERFORMANCE INDICATORS

Process performance parameter	Unit
Protein yield	%
Protein purity	% DM
Oil yield	%
Oil purity	% DM
Water consumption	kilogram / kilogram rapeseed processed
Solid waste	% DM
Wastewater	kilogram / kilogram rapeseed processed
Energy consumption	MJ / kilogram rapeseed processed
CO_2 emission	kilogram / kilogram rapeseed processed

3.1.1 MASS BALANCE PARAMETERS

The model mass balances were used to determine the recovery percentage of protein and oil from rapeseed. Product purity of both oil products and protein products was also calculated from the mass balance data.

The water consumption of the processes was calculated based on the amount of rapeseed processed. The water quality which was accounted in the models is limited purified process water (no demineralization).

Furthermore, the amount of waste streams and by-product streams were quantified based on the mass balance. Waste and by-product streams were categorized as wastewater and solid waste, respectively. Condensate water discharged by drying operations was not considered as wastewater because it does not contain any impurities.

3.1.2 ENERGY CONSUMPTION

The energy requirement of each process step was included in the models, based on specific energy consumptions of industrial unit operations. The specific energy consumption was calculated based on the power requirement

and capacity of industrial scale machines or based on available data of comparable processing industries (*see Appendix A*).

Because assumptions were made regarding the energy consumption of certain process steps, a sensitivity analysis was performed to validate if the impact of these assumptions on the total energy consumption is significant and should be taken into account. The relative sensitivity of each assumption on the total process energy consumption was determined. The calculated sensitivity indices were grouped into the four sensitivity classes shown in table 4, to evaluate the impact on the total process energy consumption [30], [31].

TABLE 4: SENSITIVITY CLASSSES [30], [31]

Sensitivity Index (SI)	Sensitivity
$0 \leq SI < 0.05$	Insensitive
$0.05 \leq SI < 0.20$	Moderate
$0.20 \leq SI < 1.00$	Highly
$SI \geq 1.00$	Extremely

If the relative sensitivity of an assumption was greater than 0.05 the effect of this assumption on the calculated overall energy consumption was considered to be significant. A relative sensitivity of 0.05 means that an increase of 10% of the assumed value, results in a total energy consumption increase of 0.5%. High and low energy consumptions are calculated for assumed energy contributors that have a significant impact on the total energy consumption, resulting in an energy consumption range.

3.1.3 CO_2 EMISSION

In this study the CO_2 emission was determined for the energy consumption during rapeseed processing. The CO_2 footprint of the whole process life cycle, e.g. chemicals, rapeseed cultivation, wastewater treatment, water treatment, packaging, transportation, was not part of this study.

Electricity and heat generation are direct contributors to greenhouse gas (GHG) emission. Electricity can be generated by fossil-fuel technologies (burning of gas or coal) or by low carbon technologies (solar, wind and nuclear energy) [32]. Emissions caused by electricity generation were based on combined cycle gas turbines (CCGT), as they are most frequently applied in industry. For industrial heat generation, fossil fuel boilers are applied most of the time. The average carbon footprints for electricity and heat generation, by CCGT's and fossil fuel boilers, are listed in table 5 [32], [33]. The mentioned carbon footprints do not only consider the major emissions caused by burning of gas, but include all emissions during the whole life cycle of electricity/heat generation [32]. Using the CO_2 emission factors listed in table 5, processing related CO_2 emissions were calculated based on the estimated electricity and heat consumption.

TABLE 5: CO_2 EMISSION FACTORS [32], [33]

Type of energy	**CO_2 emission factor** *(grams CO_2-equivalent per kWh of energy)*
Electricity *(CCGT)*	490
Heat *(Fossil fuel boiler)*	277.5

3.2 ECONOMIC EVALUATION

The goal of the economic evaluation is to gain insight in the cost-efficiency of individual unit operations and to get an estimate of the economic feasibility of the *DAP* process. The economic feasibility of the processes was evaluated by comparing the total cost price with the market value of the products.

The cost price was calculated by adding up the Operational Expenditures (OPEX) and the Capital Expenditures (CAPEX) of each processing unit operation. It should also be noted that the processes were evaluated based on major cost contributors only.

3.2.1 OPERATIONAL EXPENDITURES (OPEX)

To estimate the OPEX, cost values were assigned to the specific energy consumption of each unit operation (electricity and heat), water usage (consumption and discharge), chemical consumption and waste disposal (*see Appendix C, table 1*). Disposables like membrane replacements (of ultrafiltration and diafiltration units) were also included in the OPEX cost based on an estimation of the membrane lifetime. The OPEX were calculated per ton of rapeseed processed and per kilogram of product. Costs of labor, cleaning, maintenance and product losses invoked by cleaning were not included in the OPEX cost.

3.2.2 CAPITAL EXPENDITURES (CAPEX)

To evaluate the economic feasibility of a process, the investment costs of the unit operations were accounted for, as high investment costs can have a significant impact on the processing costs. The CAPEX cost includes depreciation costs and interest costs for the capital investment of the unit operation equipment. The CAPEX cost of a unit operation was calculated based on the investment costs of industrial scale equipment, as the economy of scale is relevant when evaluating the processing costs per ton of rapeseed processed. The industrial scale of each unit operation is described in *Appendix A*. The investment costs were multiplied by a Lang factor of 1.79 [34]. This Lang factor includes the investment costs for installation, piping and assembly of the unit operation machines. The investment costs for terrain, buildings, electrical installation, instrumentation, insurance, taxes and engineering were not included in the CAPEX calculations, because detailed process conditions based on pilot scale experiments were not provided. More detailed pilot scale experimental data on yield and performance are necessary for detailed engineering of the process.

The CAPEX costs in the model include depreciation costs and interest costs for the capital investment. The depreciation costs were based on a depreciation time of ten years and a linear depreciation of the equipment to zero. The interest costs were calculated based on the average cost of capital during the depreciation period. The interest base was set at 7% per annum. The CAPEX cost of a specific unit operation was calculated per ton of rapeseed processed and per kilogram of product, based on the yearly capacity of the specific equipment and the yearly depreciation and interest costs. In the case where the unit operation involves tanks, the specific volume and consequently investment costs were calculated based on the hydraulic residence time. The yearly capacity of the industrial scale unit operation was based on 200 operational days per year and eight hours operation per day.

3.2.3 ECONOMIC FEASIBILITY

The economic feasibility of the *DAP* process was compared with existing state of the art processes which valorise rapeseed meal. As mentioned, rapeseed meal is a side-product of the rapeseed oil refinery industry. Rapeseed meal is a commodity. The *DAP* process utilises rapeseed as a raw material. Rapeseed is a higher priced commodity than rapeseed meal. The novel protein concentrate of the *DAP* process must compete against the protein isolates produced in the benchmark processes. However, the *DAP* process side-products also have a value and are included in the economic feasibility comparison with the benchmark strategy. The market value of all the products of the *DAP* process must be high enough to bring added value to the rapeseed starting material.

The product cost price was calculated by summing of the capital, operational and raw material costs. The raw material costs of the benchmark processes (*BUR* and *TOR)* were calculated based on a commercial meal price as a starting raw material, whereas the raw material price of the *DAP* process was based on the rapeseed price (see *Appendix C, table 1*).

Because OPEX cost values are variable and have a direct impact on the final cost price of the product, a medium, low and high value for each contributor (including energy consumption ranges) was taken into account (see

Appendix C, table 1). The CAPEX calculation based on the Lang method can deviate ±50%. Therefore, also this spread was included in the cost price range calculation. The cost price was calculated in the models per kilogram of protein isolate. Because valuable side-products (oil body cream and pellet) are produced in the *DAP* process, the cost price was also calculated per kilogram of total product.

To determine the economic feasibility of the *DAP* process, the market value of the protein concentrate and the side-products was determined. The market value of each product was estimated based on a low and a high price basis. Based on these estimated market values, the range of the weighted average market value of the products was determined. The economic feasibility was subsequently evaluated by comparing the total product cost price range with the weighted average market value range. The minimum required market value to make the process economically feasible was based on the maximum cost price including a 10% margin.

3.3 MASS BALANCE CONSTRUCTION

The *DAP* process was modelled based on provided laboratory data. The *TOR* and the *BUR* processes were modelled based on process data and parameters identified from literature. A mass balance was constructed for each process based on these process parameters. In this section, the mass balance construction for each process will be elaborated on in detail.

3.3.1 PROCESS DEVELOPED BY THE UNIVERSITY OF TORONTO

PROCESS CONDITIONS AND PERFORMANCE PARAMETERS

Process conditions of the *TOR* process were identified by a literature and patent study and are summarized in table 6. These process conditions were used for constructing the mass balance and include meal to liquid ratios applied during extraction and meal washing, extraction time and pH, temperature during heat treatment, isoelectric precipitation pH, concentration factors achieved during the ultrafiltration operations and the diavolume used during diafiltration [35].

TABLE 6: PROCESS CONDITIONS OF PROCESS DEVELOPED BY THE UNIVERISTY OF TORONTO

Process step	Process conditions
Extraction	*Meal to liquid ratio*: 1:18, liquid contains 0.1 wt.% Na_2SO_3 *pH*: 12 *Time*: 30 min. [21]
Meal washing	*Meal to liquid ratio*: 1:6, liquid contains 0.1 wt.% Na_2SO_3 [21]
Mild heat treatment	Solution is heated to 60 °C for 10 minutes [22]
Ultrafiltration 1	*Concentration factor*: 3 [21]
Diafiltration 2	*Diavolume*: 3 Diafiltration buffer contains 0.1 wt.% Na_2SO_3 and has pH 12 [21]
Isoelectric precipitation	*pH*: 3.5 [21]
PVP treatment	PVP is added to a concentration of 1.0 wt.% [21]
Ultrafiltration 2	*Concentration factor*: 4 [21]
Diafiltration 2	*Diavolume*: 5 Diafiltration buffer is water [21]

TABLE 7: PROCESS YIELDS DETERMINED FOR ORIGINAL PROCESS USING COMMERCIAL RAPESEED MEAL [20]

	Solids *(%)*	**Protein** *(%)*
Starting meal	100	100
Meal residue	66.6	61.9
Precipitated protein isolate (PPI)	11.9	22.0
Soluble protein isolate (SPI)	5.7	11.0

The process yields mentioned in table 7 are of the original process of the University of Toronto, without additional membrane processing (*see Chapter 2.1.1)*. Commercial rapeseed meal was used in the process mentioned in table 7. Table 8 displays corresponding protein contents determined for the starting meal, meal residue and protein isolates [20].

TABLE 8: PROTEIN CONTENTS DETERMINED FOR ORIGINAL PROCESS USING COMMERCIAL RAPESEED MEAL [20]

	Protein content *(wt.% DM)*
Starting meal	44.7
Meal residue	41.6
Precipitated protein isolate (PPI)	82.6
Soluble protein isolate (SPI)	86.2

The most recently developed process by the University of Toronto results in lower phenolic compound contents of the produced protein isolates by additional membrane processing steps (*see Chapter* 2.1.1) [21], [22]. This process was chosen to be modeled as benchmark process. Protein yields of this process using commercial rapeseed meal as starting material are not reported in literature. Nevertheless, it is reported that the additional processing steps do not significantly affect protein recoveries of the produced protein isolates [21]. This is probably because the membranes used in the additional membrane processing steps were similar to the original membranes (identical MWCO). Therefore, protein recoveries for the selected process were assumed to be similar to those determined for the original process (table 7). However, the protein content of isolates was presumed to be slightly higher compared to the original process, as the additional processing steps result in a more extensive removal of undesired substances (fibers, phenolic compounds). As a result, the overall solids recoveries of the protein isolates were estimated slightly lower compared to the original process.

Based on the above-mentioned process conditions a mass balance model was constructed.

UNIT OPERATION SET-UP

DE-OILING

The de-oiling step comprises the commercial rapeseed de-oiling process, in which rapeseed oil and protein-rich rapeseed meal is produced. The extracted crude oil was estimated to contain 98.0 wt.% oil, based on typical crude oil purity [13]. During the de-oiling process, 42.8 wt.% of the dry-weight seed mass is recovered as crude oil [13], [36]. An average rapeseed moisture content of 8.0 wt.% was assumed [37]. Based on these figures, it was calculated that 39.4 wt.% of total rapeseed mass is recovered as crude oil in the de-oiling step.

The starting meal was reported to contain 44.7 wt.% protein on dry matter (DM) (table 8). To obtain this meal protein content, it was calculated that the starting rapeseed material has a protein content of 25.5 wt.% on DM, which is slightly above the average of 22.0 wt.% on DM.

Rapeseed protein content is inversely correlated to rapeseed oil content. Commercial rapeseed meal is reported to contain 1.0 wt.% residual oil on DM [13]. Based on these figures, the rapeseed oil content was estimated at 42.5 wt.% oil on DM, which is somewhat below the average oil content of rapeseed (45.0 wt.% DM).

The electricity, heat and hexane requirement of the rapeseed oil extraction process were based on the benchmark values (*see Chapter 1.3*).

Extraction

Sodium hydroxide (NaOH) was added during the extraction operation. In the mass balance, the required concentration of NaOH was calculated to achieve the reported pH of 12.0, based on pure water. The OPEX were determined based on industrial strength sodium hydroxide of 50.0 wt.%.

The extraction time corresponds to a hydraulic retention time (HRT) of 0.5 hours in the extraction tank. Based on the HRT and the total volume of extraction liquid, the required tank volume was calculated.

Meal separation and washing

After extraction the meal residue (pellet) is collected by centrifugation. After centrifugation another filtration step is performed to remove residual fine meal particles, to prevent clogging of the membranes in subsequent ultrafiltration and diafiltration steps. The filtration mass loss was considered to be negligible and therefore put at zero in the model.

The collected meal residue is washed to recover residual protein, which is left in the moisture of the meal. It is reported that the protein in the washed meal residue contains 61.9 wt.% of the initial meal protein (table 7). The protein content of the aqueous extract was calculated from this figure. The protein content of the wash water is assumed at 0.54 wt.% (see figure 8).

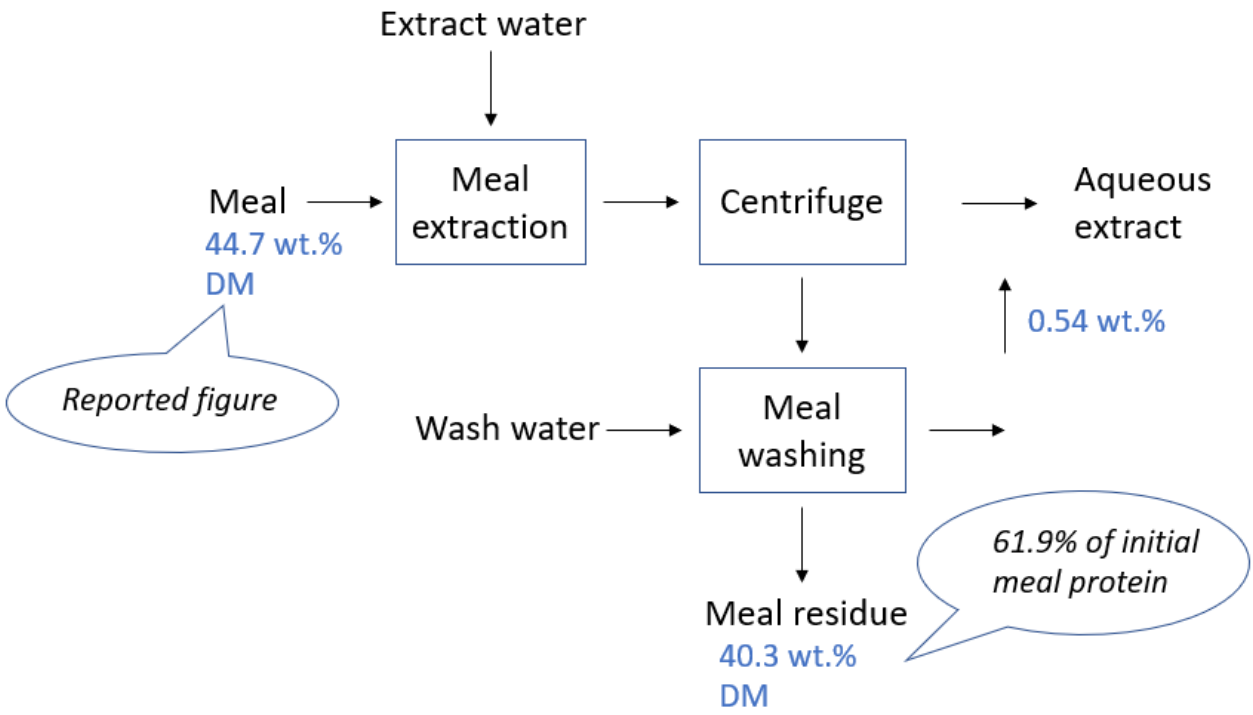

FIGURE 8: SCHEMATICAL REPRESENTATION PROTEIN MASS BALANCE OVER EXTRACTION AND MEAL WASHING STEP

The dry matter content of the meal residue after washing was reported at 66.6 wt.% of the initial total solids in the meal (table 7). The moisture content of the meal residue collected by centrifugation was estimated at 78.0 wt.%. Based on these figures, the meal residue mass recovery was calculated at 17.0 wt.%. The oil that is left in the meal after the conventional de-oiling was assumed to be completely separated off with the meal residue after centrifugation.

The downstream processing focuses on purification of the protein extraction solution from unwanted substances, such as fibers and phenolic compounds. These undesirable substances belong to the category of 'others' in the model. During this process, also some minor protein losses occur. The total protein yield of meal protein is reported at 33.0%; 22.0% and 11.0% of meal protein is recovered as precipitated protein isolate (PPI) and soluble protein isolate (SPI), respectively (see table 7). To obtain these protein yields, protein losses after extraction were distributed over all downstream unit operations

As mentioned, the process was modeled based on higher protein isolate purity because of the additional membrane processing steps. The model predicts protein isolate purities of 87.3 wt.% DM and 92.0 wt.% DM for the PPI and SPI respectively, because these isolate purities have been reported [21]. In order to obtain these isolate purities, mass losses of 'others' substances were distributed over all downstream processing unit operations.

Mild heat treatment

During the mild heat treatment, the solution is heated to 60° C for 10 minutes (table 6). The heat consumption of this operation was calculated starting at ambient temperature (*see Appendix A*).

Ultrafiltration 1

In this process step the protein solution is concentrated three times (see table 6), which corresponds to a membrane permeate recovery of 66.7 wt.% in the model.

Diafiltration 1

The required NaOH concentration of the diafiltration buffer was calculated from the reported pH, based on pure water.

In the model full permeation of diafiltration buffer was assumed, implying that the total mass of the protein solution does not change over the diafiltration operation.

Isoelectric precipitation

In the mass balance, the required concentration of acid was calculated to achieve the reported pH, based on pure water. The OPEX were determined based on hydrochloric acid of 35.0 wt.%.

The required precipitation time was estimated at a typical time of 1 hour. This corresponds to a hydraulic retention time (HRT) of 1 hour. Based on the HRT and flow of liquid entering the precipitation operation, the required tank volume for sedimentation was calculated.

The amounts of protein and others in the precipitate were calculated based on the reported purity and protein content of the precipitated protein isolate. A precipitate mass loss and moisture content of 5.0 wt.% and 83.5 wt.%, respectively, were estimated based on typical moisture content of precipitates.

PVP filtration

The resulting PVP mass loss was estimated at 0.5 wt.%, based on a typical PVP-residue moisture content of 79.0 wt.%.

Ultrafiltration 2

The reported concentration factor of 4 in this ultrafiltration operation corresponds to a membrane permeate recovery of 75.0 wt.%.

Diafiltration 2

Similar to diafiltration operation 1, full permeation of diafiltration buffer was assumed.

SPRAY DRYING 1&2

During drying the moisture content decreased to 2.0 wt.%, similar to the other process models. It was assumed that the condensate consists only of water

3.3.2 PROCESS DEVELOPED BY BURCON NUTRASCIENCE CORPORATION©

PROCESS CONDITIONS AND PERFORMANCE PARAMETERS

Process parameters of the *BUR process* were identified from a patent publication [26]. The patent publication reports data on several pilot scale experiments, in which commercial rapeseed meal is used as starting material. Extraction time, sodium chloride (NaCl) concentration of extraction liquid and the temperature of cold water used in the dilution step are the same in the reported experiments. Other process parameters, such as membrane MWCO and degree of volume reduction by ultrafiltration, show variation. Reported data of four selected experiments are summarized in table 9.

TABLE 9: PILOT SCALE EXPERIMENT DATA [26]

	Meal (kg)	NaCl solution (L)	Extraction time (min.)	NaCl conc. (M)	Protein conc. extract (g/L)	Volume after centrifugation (L)	Protein conc. after centrifugation (g/L)	Aliquot taken (L)
1	225	1500	30	0.15	19.6	nd	17.5	600
2	1200	8000	30	0.15	14.9	nd	10.4	400
3	300	2000	30	0.15	10.8	1800	8.7	all
4	300	2000	30	0.15	23.2	1772	21.7	1000
	Volume after UF (L)	**Dalton membranes**	**Protein conc. after UF (g/L)**	**Dilution factor cold water**	**Temp. cold water (°C)**	**Yield PMM of extract (%)**	**Protein content PMM (DM%)**	
1	30	3000	245	1:15	4	nd	104.1	
2	40	10,000	257	1:15	4	46	106.9	
3	55	30,000	217	1:10	4	nd	104.3	
4	52	30,000	240	1:15	4	nd	107.2	

TABLE 10: CALCULATED PROCESS CONDITIONS

	Meal to liquid ratio	Mass yield over vacuum filtration and centrifugation operation (%)	Protein concentration decrease during centrifugation[1] (g/L)
1	1:6.7	nd	2.1
2	1:6.7	nd	4.5
3	1:6.7	78.3	2.1
4	1:6.7	77.0	1.5

[1]*Protein content after centrifugation – protein content extract*

UNIT OPERATION SET-UP

DE-OILING

The commercial rapeseed de-oiling process is followed, in which crude oil and protein-rich rapeseed meal is produced. The estimated oil and mass yield are similar to those of the *TOR* process (*see Chapter* 3.3.1).

Because no data are reported on the protein content of the rapeseed meal, the rapeseed protein content and related meal protein content was set similar to the *TOR* process (*see Chapter* 3.3.1). Rapeseed oil content was calculated at 42.5 wt.% oil on DM, based on a typical residual oil content of commercial rapeseed meal of 1.0 wt.% on DM [13].

The electricity, heat and hexane requirement of the rapeseed oil extraction process were based on the benchmark values (see *Chapter 1.3*).

Extraction

The meal to liquid ratio was calculated based on the reported amounts of meal and NaCl solution used. The calculated value of all experiments is 6.7 (see table 10). The amount of NaCl was calculated based on the applied concentration (see table 9).

The extraction tank volume was calculated based on the reported extraction time (see table 9), similar to the *TOR* process.

Vacuum filtration

Reported protein extract contents vary to a large extent between the experiments. The protein concentration after vacuum filtration was 14.9 g/L. This value corresponds to an average protein concentration of pilot scale experiment 2 (see table 9).

The solids mass loss was estimated to be 20.0 wt.%. The moisture content of the separated solids was 60.0 wt.%. A full separation of oil in the solids was assumed. The protein content in the separated solids was calculated based on the reported protein content of the extract after vacuum filtration.

Centrifuge

The mass loss of the precipitated meal particles was estimated at 2.0 wt.%, based on an average total mass yield of 78.3 wt.% (liquid extract) over the vacuum filtration and centrifugation operation (see table 10). The fines moisture content was predicted at 60.0 wt.%. The protein content after centrifugation was set at 12.8 g/L. This value is based on the reported data of experiment 2. The protein content was corrected upwards by 2.4 units, based on the average protein decreases of the other experiments. The decrease of 4.5 units in experiment 2 is high compared to the other experiments (see table 10).

Filtration

The filtration mass loss of solids was set at 2.0 wt.%, because this filtration step only removes a small fraction of solids. It was assumed that no protein losses occur, because the reported protein content after ultrafiltration is high.

Ultrafiltration 1

The protein concentration after ultrafiltration of experiment 2 was reported at 257 g/L. The membrane permeate recovery was calculated at 90.0 wt.%, based on a reported ultrafiltration volume reduction of 400 to 40 litre (see table 9). However, to reach the target concentration of 257 g/L the recovery was adjusted to 94.9 wt.% in the model. No protein losses were assumed in the permeated liquid, in order to reach the target protein concentration with achievable membrane recovery rate.

Dilution + precipitation

Experiment 2 reports a PMM protein yield of 46.0 wt.%. This yield is defined in the mass balance as the ratio between kilogram protein recovered after sedimentation and kilogram protein extracted after ultrafiltration. The corresponding dilution factor of 1:15 was used.

A sedimentation yield of 3.7 wt.% was estimated, to reach a typical sediment moisture content of 80.0 wt.%. Because of the high purity of the protein isolate, no pollution (i.e. 0 wt.% 'others') was considered to be in the sediment.

ULTRAFILTRATION 2

At this concentration step the protein solution is concentrated to 200-300 g/L, as reported in the general patent description [26]. In the model this corresponds with a membrane permeate recovery of 96.0 wt.% and no protein losses in the permeated liquid. The residual 'others' fraction is almost completely removed in the permeated liquid, to reach the high protein isolate purity reported.

SPRAY DRYING 1&2

During drying the moisture content was decreased to 2.0 wt.%, similar to the other models. It was assumed that the condensate consists of water only.

3.3.3 DESIGNED AQUEOUS EXTRACTION PROCESS

PROCESS CONDITIONS AND PERFORMANCE PARAMETERS

Process conditions that were used for constructing the mass balance are listed in table 11. These included the ratio of solids to water applied during soaking, soaking time/pH and the applied diavolume during diafiltration.

TABLE 11: PROCESS CONDITIONS OF DESIGNED AQUEOUS EXTRACTION PROCESS

Process step	Process conditions
Soaking	*Ratio solids to water* = 1:8 *Soaking time:* 4 hours *pH:* 9
Diafiltration	*Diavolume* 6, diafiltration buffer is 8 mM salt solution Followed by: *Diavolume*: 1, diafiltration buffer is water

Laboratory data were used to validate the mass balance (*see Appendix D, table 1*). The data included the composition (protein, oil and moisture content) of the main stream and side-products as well as the composition of the dehulled rapeseed material.

The mass balance of the extraction was reconstructed based on the provided laboratory data (*see Appendix D, table 2)*. In the reconstruction it was assumed that no oil losses occur during diafiltration, because oil is extracted in the form of oil bodies. Oil bodies typically have a size in the range of 0.2-2.5 µm or even bigger [38]. The membrane used in the diafiltration operation has a MWCO of 5 kDa and should completely retain these oil body molecules. Because no laboratory data were available on oil content in the liquid after the screw press operation, the oil content was estimated at 4.1 wt.%. This estimation was based on the oil content of the separated solids, the oil balance in the centrifuge separation (oil content pellets and cream) and the oil content of the dehulled rapeseed. For a detailed analysis of the oil balance the oil content has to be determined.

The calculated mass balance in- and output of the extraction operation (soaking and blending operations followed by screw press) and the centrifugation operation showed differences on mass as well as on protein, moisture and oil (*see Appendix D, table 2*). These differences are probably a result of laboratory scale experimental losses and will hereafter be referred to as unaccounted losses.

The by-product mass loss percentage of each unit operation was calculated (*see Appendix D, table 3*). A detailed description of the method for calculating the efficiencies of each unit operation is explained in the following

section called *Unit operation set-up* (see below). A larger scale model was constructed, based on the composition and calculated mass loss percentages (of waste and side-product streams) of the laboratory experiment.

The distribution of unaccounted losses among the product streams was unknown and therefore the unaccounted losses of the extraction operation in the larger scale model were added to the solids. The unaccounted losses of the centrifugation step were added to the pellets. This resulted in a higher fraction of solids and pellets as well as a slightly different composition of the solids and pellets. For that reason, the yield efficiencies of protein and oil in the results section are calculated from the analysis data of the samples and volumes of the side-products as determined in the laboratory experiment, excluding the unaccounted losses.

Unit operation set-up

Dehulling

The composition of the dehulled material was measured in the laboratory. The amount of removed hull was determined to be 25.0 wt.% of total rapeseed hull.

The average moisture content of the initial rapeseed was 8.0 wt.%. The mass loss due to dehulling was determined as 4.1 wt.%. This value was based on an average literature hull content (16.6 wt.% on DM) and the above mentioned hull removal rate (25.0 wt.%) [39]. Hull moisture content was set similar to the moisture content determined for the dehulled rapeseed material. Because laboratory data provided no information on hull composition, hull oil and hull protein content were based on literature values with an average of 13.9 wt.% on DM and 18.4 wt.% on defatted DM, respectively [39]. Based on oil and protein component balances over the dehulling step, the oil and protein content of the initial rapeseed were calculated to be 41.9 wt.% on DM and 19.1 wt.% on DM, respectively. Based on the moisture balance over the dehulling step an assumption was made on water losses. These moisture losses (62.3 wt.% of total rapeseed moisture) were also included in the larger scale model.

Soaking and blending

NaOH was added during the soaking operation. In the mass balance, the required concentration of NaOH to achieve a pH 9.0 was calculated similar to the extraction step in the *TOR* process. The OPEX were determined based on industrial strength sodium hydroxide of 50.0 wt.%.

The hydraulic retention time (HRT) and resulting tank volume were calculated similar to the *TOR* process.

Screw press

As mentioned, the percentage of lost mass and composition of solids at the screw press operation included the mass balance differences.

Centrifugation

The percentage of lost mass and composition of the oil body cream at the centrifugation step was based on laboratory data. As mentioned, the percentage of lost mass and composition of the pellet included the mass balance differences.

Filtration

A filtration operation was included in the model. This filtration step was performed at laboratory scale. As there were no data available regarding this process step, the mass losses were set to zero.

Diafiltration

The amounts of salt and water added at the diafiltration operation were calculated from the process data listed in table 11. The amount of protein and ‘others’ lost in the permeated liquid of the diafiltration unit were determined based on the analysed composition of the subnatant and the final product.

In the model, full permeation of diafiltration buffer was assumed, implying that the total mass of the protein solution does not change over the diafiltration operation.

ULTRAFILTRATION

An ultrafiltration step was added to the model, to compensate for high drying energy requirement, which would occur if diafiltration were to be directly followed by freeze-drying. The protein solution was concentrated to 226 g/L, similar to protein contents achieved in the ultrafiltration pre-concentration step of the *BUR* process (see section 3.3.2). It was assumed that no protein, oil and 'others' losses occurred in this step by using the same MWCO membrane as in the diafiltration step.

FREEZE-DRYING

Water removal was set to 65.6 wt.%, to a reach a protein concentrate moisture content of 2.0 wt.%. This water removal percentage was lower compared to the laboratory-scale experiment (*see Appendix D, table 3*), because an ultrafiltration concentration step was included in the model. It was assumed that the condensate consists only of water.

4 RESULTS AND DISCUSSION

4.1 PROTEIN YIELD

A high protein yield and recovery contributes to the economic feasibility of a process. The protein mass distribution of the laboratory experiment, excluding the unaccounted losses, is illustrated in figure 9.

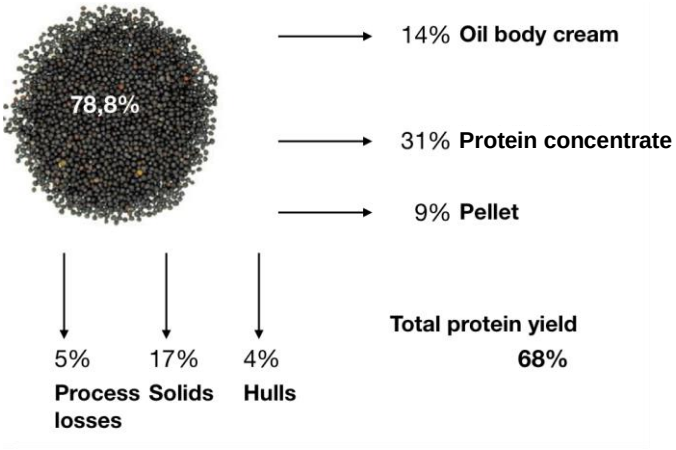

FIGURE 9: LAB SCALE PROTEIN MASS DISTRIBUTION *DAP* PROCESS

Excluding the unaccounted losses, 78.8% of total rapeseed protein was recovered accounting products, by-products and process losses (wastewater). The unaccounted protein loss was 21.2% of the total rapeseed protein content, however the distribution of these losses is unknown (*see Chapter* 3.3.3) and therefore not included in figure 9.

The total protein recovery was 53.5% of total rapeseed protein, including the protein concentrate, oil body cream and pellet products. The total protein yield of the products excluding the unaccounted losses was calculated at 68.0% (53.5% total protein recovery divided by 78.8% total accounted protein). Similarly, the protein yield of only the protein concentrate was calculated to be 39%, excluding unaccounted losses (31% recovery divided by 78.8% total accounted protein).

Overall protein yield and protein isolate purities as calculated from the mass balance models are listed in table 12.

TABLE 12: OVERALL PROTEIN YIELD AND PURITY OF PROTEIN ISOLATE PRODUCTS

	DAP process	TOR process		BUR process	
Protein yield *(% protein of total rapeseed protein processed)*	39 (68)[1]	33		20	
		PPI	**SPI**	**PPI**	**SPI**
Protein isolate purity *(protein wt.% on DM)*	67	87	92	100	100

[1] *Total protein yield from rapeseed including side-products*

Overall, protein yields of the protein concentrate and isolates varied between 20% and 39% of total rapeseed protein. The *DAP* process has the highest protein yield (39%), whereas the *BUR* process has the lowest protein yield (20%). The *TOR* process has an average protein yield of 33%.

When including the proteins present in the oil body cream and pellet side-products, the total protein recovery of the *DAP* process is even higher; 68%. The protein yields of the side-products cream and pellet comprised 17.7% and 11.4% of total rapeseed protein, respectively. It should be noted that valorisation of the cream and pellet side-products increases the value of these products, justifying their contribution to the total protein yield.

The lower protein yields of the *BUR* and *TOR* process can be explained by the lower extraction efficiency due to the limited solubility of the proteins in the commercial rapeseed meal. Proteins present in the commercial rapeseed meal have limited solubility, due to the harsh processing conditions (heat and pressing steps) that are applied in the commercial rapeseed de-oiling process [2]. The use of intact rapeseed and mild extraction conditions applied in the *DAP* process lead to higher protein extraction yields.

Protein isolate purities varied to a larger extent between the processes. The *TOR* and the *BUR* process both produce two different protein isolates: a precipitated protein isolate (PPI) and a soluble protein isolate (SPI). Both benchmark processes employ intensive downstream processing to obtain high protein isolate purity (see table 12). Besides, extracted rapeseed proteins are separated into two different protein fractions, which have different functional properties. Separation of the protein into two isolates increases the uniformity of functional properties of the isolate products, which in turn leads to a broad applicability in the food industry. This strategy requires extra purification steps to extract the separate fractions. In contrast the *DAP* process is able to efficiently extract both protein fractions in one concentrate without harsh conditions. This concentrate has a lower protein purity of 67 wt.% on DM. However, due to the mild conditions applied during the fractionation process and the absence of the commercial de-oiling process, techno-functional properties such as interfacial activity might be superior to the protein isolates produced in the benchmark processes.

4.2 OIL YIELD

Because oil is the main product of rapeseed processing, oil yield has also been considered in the process comparison. In figure 10, the oil mass distribution of the laboratory experiment of the *DAP* process is illustrated, excluding unaccounted losses (similar to the protein mass distribution, *see Chapter 4.1*). 97.1% of total rapeseed oil was accounted in products, by-products and waste.

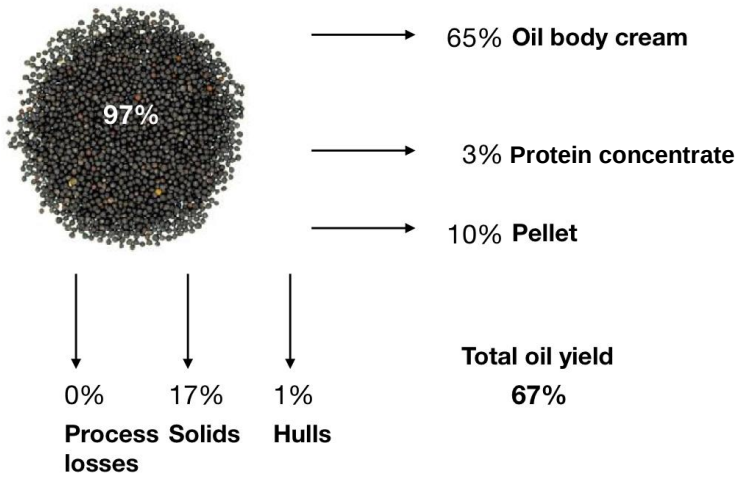

FIGURE 10: LAB SCALE OIL MASS DISTRIBUTION *DAP* PROCESS

The oil yield of the oil body cream excluding unaccounted losses was calculated at 67%, similar to the protein yield calculation (65.1% oil recovery in oil body cream divided by 97.1% total accounted oil). The total oil recovery including oil body cream and pellet side-products was calculated at 81%, excluding the unaccounted losses.

Oil yields and purities of the *DAP* process and the conventional oil extraction process are summarized in table 13.

TABLE 13: OIL YIELD OF TOTAL RAPESEED PROCESSED AND OIL PURITY

	DAP process	Commercial oil extraction
Oil yield *(% oil of total rapeseed oil processed)*	67 (81)[1]	99
Oil purity *(oil wt.% on DM)*	82	98

[1] *Total oil yield from rapeseed including side-products*

Oil yields achieved in the commercial oil extraction process are higher compared to the *DAP* process. This is because high oil extraction efficiencies are achieved in the commercial rapeseed de-oiling operation, which focuses on oil extraction. Achieved oil purities of in the commercial oil extraction process are also high, because hexane extraction very selectively extracts oil. The high purity rapeseed oil produced in the benchmark processes, has proven market potential in the food and biodiesel industries.

The oil extraction efficiency and achieved oil purity of the *DAP* process are lower, due to the mild fractionation applied. Around 67% of total rapeseed oil is recovered in the oil body cream. However, oil is extracted in the native form of oil bodies, which have natural emulsifying characteristics. Besides, oxidation of oil is prevented because of the external protein surface layer that surrounds the oil bodies [40]. The application of the oil body cream side-product in the food, pharmaceutical or cosmetics industry has to be investigated to determine its market potential.

4.3 WATER CONSUMPTION

Water consumption has a large impact on the sustainability of a production process, because water resources are becoming scarce due to climate change [41]. All three processes consume considerable amounts of water during extraction and purification of the extracted protein solution (downstream processing). In figure 11, water consumption attributed to extraction and downstream processing is depicted for all processes.

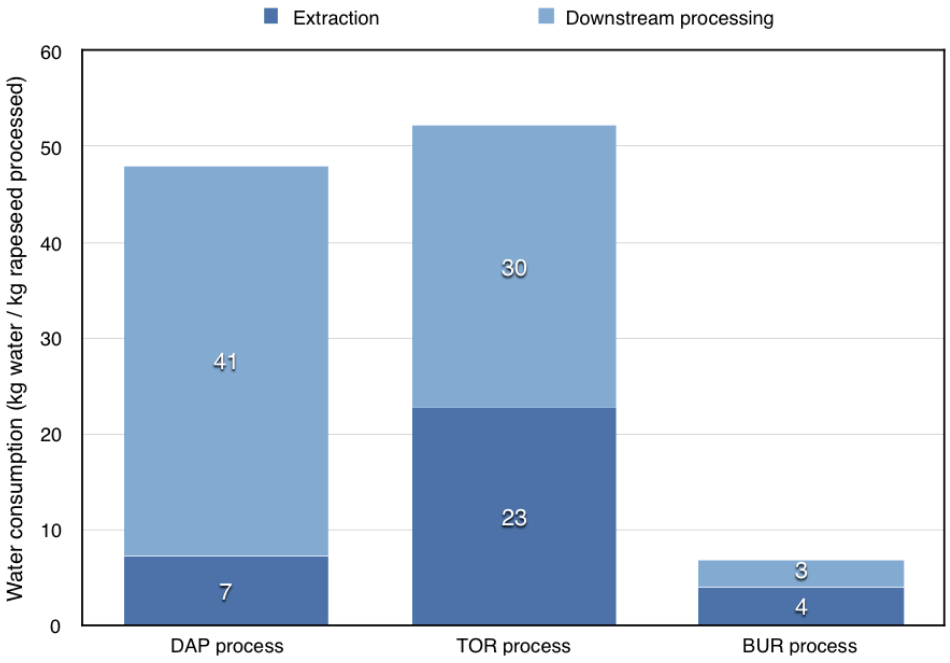

FIGURE 11: WATER CONSUMPTION PER KILOGRAM OF RAPESEED PROCESSED

The *TOR* process has highest overall water consumption of 52 kilogram water per kilogram of processed rapeseed, with remarkable high water consumption during the extraction compared to the other processes. The *DAP* process requires in total 48 kilogram of water per kilogram of processed rapeseed with the water consumed during downstream processing as the main contributor to the overall water consumption. The *BUR* process reports the lowest overall water consumption of 7 kilogram water per kilogram per kilogram of rapeseed processed. In the *BUR* process least water is consumed during both extraction and downstream processing. Probably the water efficiency of the pilot scale *BUR* experiment is more optimized.

4.3.1 WATER CONSUMPTION DURING EXTRACTION

The water consumption of the extraction process influences the protein yield. This is because higher liquid to solid ratios during extraction, also lead to higher protein extraction yields due to concentration limitation effects. High yields achieved by high water ratios will in turn lead to high downstream processing costs. Therefore, an optimum ratio has to be determined. In table 14, protein yields (over the extraction step) are related to the water consumption by dividing the protein extraction yield by the extraction water consumption per kilogram rapeseed processed. A high protein extraction yield per kilogram extraction water is an indicator for an efficient protein extraction process.

TABLE 14 PROTEIN EXTRACTION YIELDS RELATIVE TO EXTRACTION WATER CONSUMPTION

	DAP process	TOR process	BUR process
Protein extraction yield *(% protein of dehulled rapeseed/meal processed)*	66[1,2]	38	20
Protein yield divided by extraction water consumption	9.0	1.7	4.9

[1] *The dehulling operation is not included in the calculated extraction yield*

[2] *Based on laboratory scale experiment*

As shown table 14, the *DAP* process has the highest protein extraction yield in relation to the extraction water used, due to the absence of the harsh oil extraction process. The absence of the commercial de-oiling process likely results in an increased solubility of rapeseed proteins, leading to a higher extraction yield. The protein yield may even be higher, because the amount of unaccounted protein losses at the extraction (soaking and blending operations followed by screw press) were significant (16% of total rapeseed protein). Pilot scale experiments are necessary to validate the protein extraction yield.

4.3.2 WATER CONSUMPTION DURING DOWNSTREAM PROCESSING

During extraction undesirable substances can be co-extracted, which have to be removed in the downstream processing. Dilution and diafiltration steps are applied to remove these substances which generally require large amounts of water.

Noteworthy is that the water consumption during downstream processing in the *BUR* process is low (3 kilogram/kilogram rapeseed processed) compared to the other processes, even though the produced protein isolates are of high purity (100 wt.% protein on DM). This is maybe due to the pre-concentration by ultrafiltration prior to dilution resulting in a more effective washing, in combination with less co-extracted impurities due to the saline extraction.

4.4 ENERGY CONSUMPTION AND PROCESSING RELATED CO_2 EMISSION

Energy consumption of the processes has been considered in the overall comparison, because large energy requirements decrease process sustainability and can lead to high operational costs.

4.4.1 SENSITIVITY ANALYSES

Table 15, 16 and 17 show relative sensitivity values of the energy consumption of each processing step for each process. The relative sensitivity indices are ranked as insensitive, moderately sensitive, highly and extremely sensitive based on the sensitivity classes mentioned in Chapter 3.1.2, table 4.

TABLE 15: RELATIVE SENSTIVITY INDICES OF PROCESS STEPS WITH REGARD TO ENERGY CONSUMPTION IN *TOR PROCESS*

De-oiling	**Extraction**	**Centrifugation**	**Filtration**	**Meal washing**	**Heating**	**Ultrafiltration 1**
0.08	0.00	0.00	0.00	0.00	0.25	0.00
Moderately sensitive	Insensitive	Insensitive	Insensitive	Insensitive	Highly sensitive	Insensitive
Diafiltration 1	**Drying 1**	**PVP filtration**	**Ultrafiltration 2**	**Diafiltration 2**	**Drying 2**	
0.00	0.10	0.00	0.00	0.00	0.56	
Insensitive	Moderately sensitive	Insensitive	Insensitive	Insensitive	Highly sensitive	

In the *TOR* process the drying and heating steps and the commercial oil extraction process mostly influence the total energy consumption. The specific energy consumption of the heating step and the commercial oil extraction process were based on verified values (*see Appendix A*). The energy consumption of the drying step was based on the average energy consumption of industrial spray-dryers. However, the energy consumption of a spray-dryer depends on various factors, including initial and final moisture content as well as the feed flow [42]. For that reason, a spread of ±10% of the average spray drying energy requirement was applied for calculating the overall energy consumption range.

TABLE 16: RELATIVE SENSTIVITY INDICES OF PROCESS STEPS WITH REGARD TO ENERGY CONSUMPTION IN *BUR PROCESS*

Deoiling	**Extraction**	**Vacuum filtration**	**Centrifugation**	**Filtration**
0.49	0.00	0.00	0.01	0.00
Highly sensitive	Insensitive	Insensitive	Insensitive	Insensitive
Ultrafiltration 1	**Dilution+sedimentation**	**Drying 1**	**Ultrafiltration 2**	**Drying 2**
0.00	0.14	0.17	0.00	0.18
Insensitive	Moderately sensitive	Moderately sensitive	Insensitive	Moderately sensitive

In the *BUR* process the assumption made regarding the energy requirement of the dilution step has a significant effect on the total energy consumption of the process. The energy consumption of the dilution step depends on the water source temperature and was calculated assuming a water source temperature of 10 °C. However, groundwater temperature depends on the location and season. Because the water temperature has a significant effect on energy usage, it is most likely that industries processing rapeseed using the *BUR* method will not be located in areas where the water temperature is high. Therefore, a groundwater temperature range of 9-11 °C, corresponding to moderate climate conditions, was selected for calculating the overall energy consumption range [43].

The drying steps of the *BUR* process also have a significant effect on the total process energy consumption. Similar to the *TOR* process, a 10% spread in the spray drying energy requirement was taken into account for calculating the overall energy consumption range.

TABLE 17: RELATIVE SENSTIVITY INDICES OF PROCESS STEPS WITH REGARD TO ENERGY CONSUMPTION IN *DAP PROCESS*

Dehulling	**Soaking**	**Blending**	**Screw press**	**Centrifugation**
0.01	0.00	0.02	0.00	0.02
Insensitive	Insensitive	Insensitive	Insensitive	Insensitive
Filtration	**Diafiltration**	**Ultrafiltration**	**Freeze-drying**	
0.00	-0.10	-0.02	0.82	
Insensitive	Moderately sensitive	Insensitive	Highly sensitive	

In the *DAP* process, the energy consumption of the diafiltration operation is moderately sensitive. The assumed value comprises the applied average flux, which results in a specific energy consumption. A minimum and maximum value for the diafiltration flux was applied for calculating the overall energy consumption range. The estimated flux (14.8 L/m^2*bar) was close to the highest clean water flux (15 L/m^2*bar), reported by the membrane manufacturer (*see appendix A*), because the average protein content at diafiltration was low. An energy consumption range was calculated based on a minimum flux of 10 L/m^2*bar and the maximum flux of 15 L/m^2*bar (equal to the clean water flux).

The freeze-drying operation also has a significant effect on the total energy consumption of the *DAP* process. The energy consumption of freeze-drying depends on the specific moisture extraction rate (SMER, *see Appendix A*). The exact SMER value has to be determined in a pilot-scale set-up. The specific energy consumption of freeze-drying in the model was estimated based on a relatively high SMER of 0.4 kg water per kWh. However, lower SMER values can occur [44]. For that reason, a minimal SMER value of 0.35 kg water/kWh and a maximum SMER value of 0.4 kg water/kWh were considered in the overall process energy consumption range.

4.4.2 COMPARISON DESIGNED AQUEOUS EXTRACTION PROCESS WITH BENCHMARK PROCESSES

In table 18, the calculated total energy consumption ranges of the processes are shown. The *TOR* process has the highest overall energy consumption and is likely not optimized regarding energy efficiency. The *DAP* process has a slightly lower energy requirement compared to the benchmark *BUR* process.

TABLE 18: OVERALL ENERGY CONSUMPTIONS OF THE PROCESSES

	DAP process	**TOR process**	**BUR process**
Total energy consumption *(Wh / kg rapeseed processed)*	484-566[1]	3726-4249	623-701
Total energy consumption *(MJ / kg rapeseed processed)*	1.74-2.04[1]	13.4-15.3	2.24-2.52

[1] *DAP process including the ultrafiltration concentration step prior to freeze-drying*

In figure 12, 13 and 14 the energy distribution of the processing steps is illustrated.

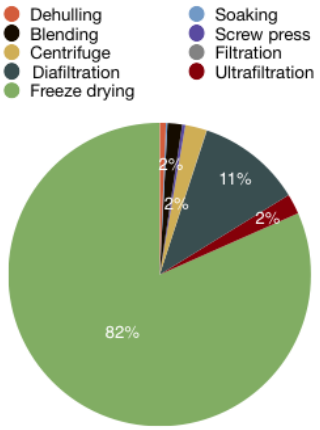

FIGURE 12: ENERGY USAGE *DAP* PROCESS

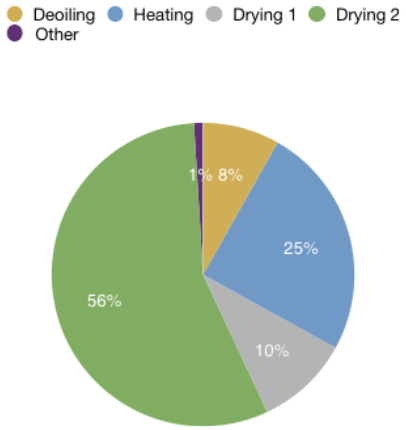

FIGURE 13: ENERGY USAGE *TOR* PROCESS

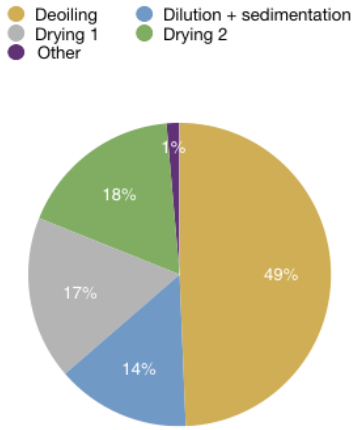

FIGURE 14: ENERGY USAGE *BUR* PROCESS

The freeze-drying step of the *DAP* process contributes most to the total energy consumption. Freeze-drying is not often applied in large-scale industrial operation as drying method because freeze-drying requires a lot of energy. Alternative drying methods like spray drying are more widely adopted. The relative energy consumptions of the diafiltration and ultrafiltration operations also significantly contribute to the total energy consumption of the *DAP* process. This is due to the large amount of water processed in combination with the tight ultrafiltration membrane (5 kDa) used with a low average flux. The diafiltration was reported to use a diavolume of seven times the subnatant resulting from the centrifuge operation. Because the subnatant was not concentrated prior to diafiltration large amounts of diafiltration buffer are required.

Drying steps contribute largely to the overall energy requirement of both benchmark processes. Drying operations generally have high energy consumption because water is evaporated. Industrial spray dryers require on average 4.87 MJ to evaporate one kg of water [45]. To minimize the overall energy consumption of water removal, it is crucial to concentrate the solution prior to drying. Concentration of protein solutions is often done by ultrafiltration (*see Appendix A*) [46]. Ultrafiltration requires energy to transfer water across a membrane. The required pumping energy increases when high protein concentration (i.e. high water removal) is targeted. High protein concentration will inevitably lead to a lower flux. Nevertheless, the energy required for water removal by ultrafiltration is much lower compared to spray drying operations (4.87 MJ per kilogram water). Even at a low ultrafiltration flux (1 l/m^2*bar) the specific energy requirement of 68.4 kJ per kilogram of water removed is significantly lower than spray drying.

In the *BUR* process the protein solution was concentrated by ultrafiltration to a protein content of over 200 g/L prior to drying. In the *TOR* process a solution having a much lower protein concentration (14 g/L) is directly dried. This results in a high energy consumption of the *TOR* process. Furthermore, the mild heat treatment applied in the *TOR* process contributes largely to the overall energy requirement of the process, because of the large volume of water that is heated. The mild heat treatment even consumes more energy than the entire commercial de-oiling process.

In the *BUR* process the dilution step is a large contributor to the total energy consumption, in addition to the drying steps and the de-oiling operation. This is because in the dilution step around three kilograms of cold water (4 °C) are required per kilogram of rapeseed processed. The water has to be cooled down to 4 °C, whereas the water source has a temperature of around 10 °C. This requires a lot of energy taking into account the low energy efficiency of industrial refrigerators used for the cooling operation of the water source. Considering the overall energy consumption, the commercial de-oiling process consumes most energy in the whole production chain

from rapeseed to protein isolate in the *BUR* process. This is because in the *BUR* process the other processing steps (for example the drying) do not have excessively large energy requirements, compared to the *TOR* process.

4.4.3 PROCESSING RELATED CO_2 EMISSION

The type of processing energy used has an effect on the CO_2 emission. The applied method of downstream processing influences this CO_2 emission, e.g. heat required for removal of process water has another CO_2 footprint than electricity required for membrane concentration. The CO_2 footprint of heat generation is lower than the CO_2 footprint of electricity generation (*see Chapter* 3.1.3). In table 19, CO_2 emission ranges based on the calculated energy consumptions range are listed.

TABLE 19: PROCESSING RELATED CO_2 EMISSION

	Energy *(MJ / kg rapeseed processed)*	**Electrical energy**	**Heating energy**	**Processing related CO_2 emission** *(kg CO_2-eq/kg rapeseed processed)*
DAP process	1.74-2.04	100%	0%	0.24-0.28
TOR process	13.4-15.3	5%	95%	1.1-1.2
BUR process	2.24-2.52	24%	76%	0.20-0.23

The processing related CO_2 emission of the *BUR* process is slightly lower compared to the *DAP* process, even though the *BUR* process has a higher energy consumption. This is probably attributed to the high percentage of electrical energy in the *DAP* process, which is caused by the freeze-drying step.

The CO_2 emission of the *TOR* process is the highest due to the large energy requirement of the process.

4.5 WASTE STREAMS

The quantity of waste or by-product streams produced during the fractionation process is important for resource efficiency and environmental impact. Although by-products are often reused or refined, the percentage loss of rapeseed raw material is crucial for evaluating the sustainability of the process.

4.5.1 SOLID WASTE AND BY-PRODUCT STREAMS

During the extraction process part of the proteins and rapeseed substances is not extracted and ends up in the by-product streams, e.g. hulls, solids, and meal residue. Besides, other molecules are co-extracted, which are removed by addition of dilution and diafiltration steps and end up in the in wastewater. The amount of these losses can be expressed as dry matter percentage of total processed rapeseed dry matter.

In the mass balance of the *DAP* process, which is based on laboratory experiment, 88.6% of the rapeseed dry matter was accounted in the product and by-product streams. The dry matter recovery of 88.6% was recalculated to 100% based on equal distribution, for comparison with the benchmark processes (similar to the oil and protein yield calculation). The unaccounted losses amounted 11.4% of the total rapeseed dry matter and could be either product or by-product. The calculated dry matter percentages may therefore change when the unaccounted losses are defined.

The calculated dry matter distribution for each process including products/side-products, wastewater losses and solids losses is shown in table 20.

TABLE 20: DRY MATTER DISTRIBUTION

	Product *(% DM of total DM processed)*	**Wastewater loss** *(% DM of total DM processed)*	**Solids loss** *(% DM of total DM processed)*
DAP process	60[1]	9	31[2]
TOR process	52[3]	9	39[4]
BUR process	48[3]	7	45[4]

[1]*Protein concentrate and side-products: oil body cream and pellet*

[2]*Solids, hulls*

[3]*Protein isolate, crude oil*

[4]*Meal residue*

The product dry matter recovery is highest in the *DAP* process; 60% of total DM. The harsh commercial oil extraction process in the conventional extraction route, which focuses on high yielding oil extraction, results in a high waste generation. Also, the quality of the resulting meal residue is low, because meal protein has been extracted to a large extent and the remaining protein in the meal residue is probably denatured due to the harsh oil extraction conditions.

4.5.2 WASTEWATER

Wastewater production has been considered in the process evaluation because wastewater treatment has a significant environmental impact due to sludge production and disposal problems. Wastewater treatment also invokes high energy consumption.

Wastewater production is directly related to water consumption. In the processes a lot of water is used as processing water for extraction and downstream purification (see section 4.3). Most of this processing water will be discharged as wastewater. In table 21, the amount of wastewater produced per kilogram of rapeseed processed is listed.

The wastewater to product ratio is a commonly used parameter for evaluating the water consumption of a production process [47]. The amount of wastewater produced per kilogram of product dry matter was selected as parameter for comparing the processes. The dry matter content was used because the *DAP* process side-products (oil body cream and pellet) have a higher water content compared to the products of the *TOR* and *BUR* benchmark processes.

TABLE 21: WASTEWATER GENERATION IN PROCESSES

	DAP process	**TOR process**	**BUR process**
Wastewater *(kg per kg rapeseed processed)*	46.2	48.9	6.1
Wastewater *(kg per kg product DM)*	94.3[1]	101.9	13.8

[1]*Based on laboratory scale experiment*

The specific wastewater production of the *DAP* process and the *TOR* process is high compared to the *BUR* process. The product yield of these processes is not high enough to compensate for the high water consumption (see section 4.3).

The calculated product yield of the *DAP* process may be higher when the unaccounted process losses are defined, because the unaccounted losses could comprise products. This would result in a slightly lower specific wastewater production. Nonetheless, improvement of the water consumption of the *DAP* process is necessary to lower the high specific wastewater production.

4.6 ECONOMIC EVALUATION

The economic feasibility of the protein extraction process was determined by a cost price calculation. The cost price has to be significantly lower than the estimated market value of products to make the process economically feasible.

4.6.1 COST PRICE COMPARISON

The cost price of the production process was calculated by adding the OPEX cost to the CAPEX cost and raw material cost. The cost price build-up of each process is shown in figure 15.

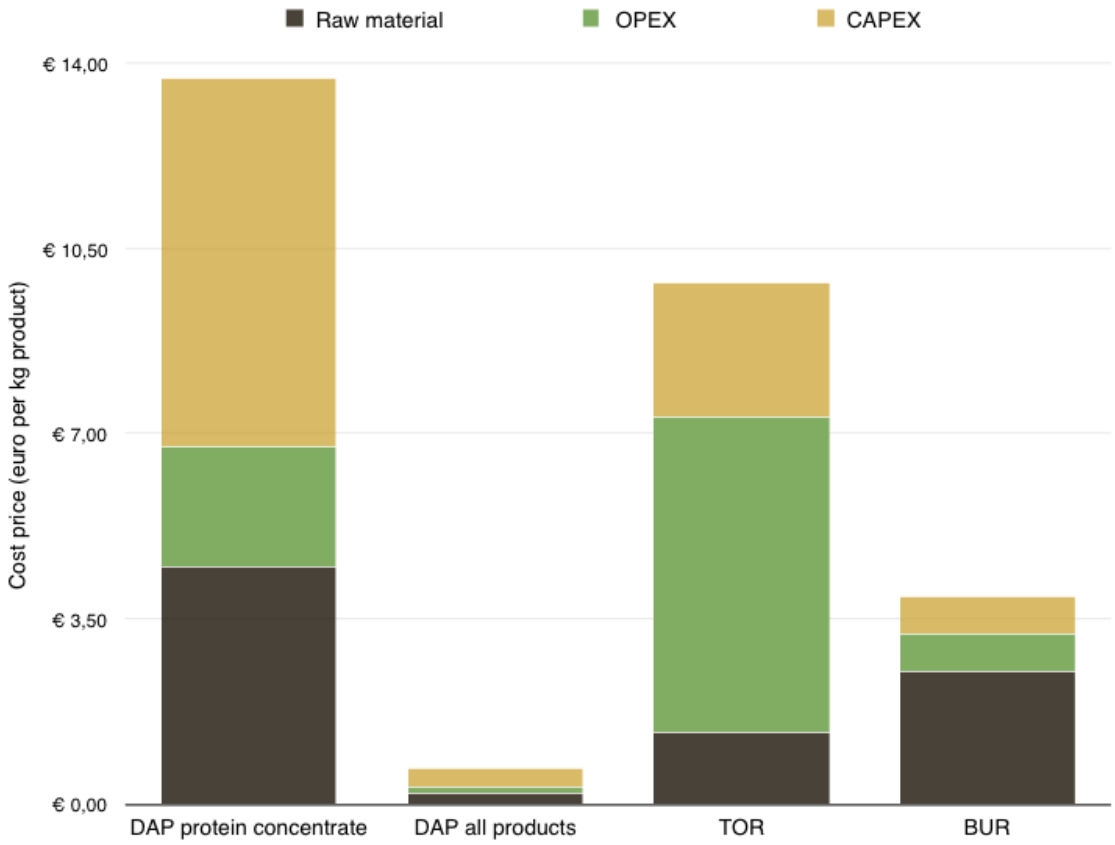

FIGURE 15: COST PRICE BASED ON MEDIUM COST VALUES

In this figure the OPEX costs were based on medium cost values (*see Appendix C*). The CAPEX cost calculation base were the investment costs of industrial capacity machines (*see Appendix A*). The raw material cost of the *BUR* and *TOR* process was calculated from a commercial meal price of €197 ($227) per ton Hamburg FOB [7]. The raw material price of the *DAP* process was based on the rapeseed price of €374 ($430) per ton Hamburg CIF [7].

The cost price of the *DAP* process is split into two bars: *DAP protein concentrate* and *DAP all products*. The cost price of *DAP protein concentrate* is based on the dried protein concentrate only. The cost price of *DAP all products* also includes the oil body cream and pellet side-products. All products produced by the *DAP* process combined, have a low average cost price. The major reason of this low price is the inclusion and valorisation of the wet side-products in the cost price. The cost price of raw material of the *DAP protein concentrate* is higher compared to the *BUR* and *TOR* process, as intact rapeseed has a higher market value per kilogram than rapeseed meal used in *BUR* and *TOR* process. The *TOR* process has a higher yield compared to the *BUR* process and therefore a lower raw material cost price.

The CAPEX costs of *DAP* process are relatively high compared to *BUR* and *TOR* process (see figure 15) and attributed to the high investment cost of the freeze-drying operation accounting for 90% of the total CAPEX costs.

The OPEX cost price of the *DAP protein concentrate* is slightly higher than the OPEX cost price of the *BUR* process but significantly lower than the OPEX cost price of the *TOR* process. To get a better understanding of the reasons behind the differences in OPEX cost, the OPEX cost distribution for each process is illustrated in figure 16, 17 and 18.

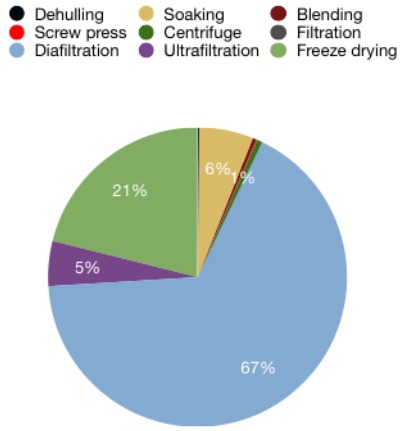

FIGURE 16: OPEX *DAP* PROCESS

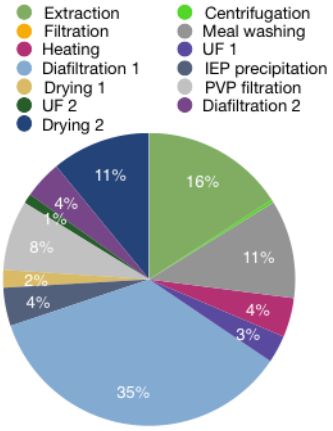

FIGURE 17: OPEX *TOR* PROCESS

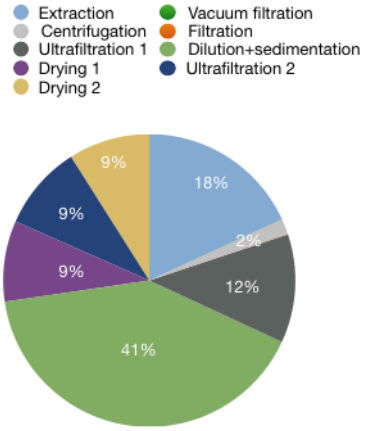

FIGURE 18: OPEX *BUR* PROCESS

The main contributor to the OPEX costs of the *DAP* process is the diafiltration operation. The diafiltration OPEX costs are high because of the high water usage and consequently wastewater discharge costs. The freeze-drying operation also contributes significantly to the OPEX costs, because of the high electricity consumption of the freeze-drying process.

The OPEX costs of the *TOR* process are high compared to the *BUR* and *DAP* process (see figure 15). This is mainly influenced by the consumption of chemicals and the high water consumption at the extraction and diafiltration operation. Also, the drying operation contributes significantly to the total OPEX cost, because a high water volume is evaporated.

The OPEX cost distribution of the *BUR* process shows that the cold water dilution step is significantly contributing to the costs, mainly due to the cost of cooling and the high water consumption. The high amount of extraction water and chemicals used also significantly contributes to the OPEX cost.

4.6.2 ECONOMIC FEASIBILITY

The economic feasibility is largely influenced by the cost price. Because the values for the OPEX costs are variable and have a direct impact on the final cost price of the product, a high and low value for each contributor was taken into account (*see Appendix C*). The CAPEX calculation deviates ±50% based on the Lang method. Therefore, also this spread was included in the cost price range, which can be found in table 22.

TABLE 22: COST PRICE RANGES INCLUDING OPEX AND CAPEX COST VARIATION

	DAP process		TOR process	BUR process
Cost price *(euro per kg product)*	0.47-0.91[1]	9.55-18.59[2]	7.61-12.99	3.33-4.73

[1]*Including protein concentrate only*

[2]*Also including side products: oil body cream and pellet*

The protein isolates produced in the *BUR* process and the *TOR* process have cost prices in the range of 3.33-4.73 and 7.61-12.99 euro per kilogram, respectively. The market value of rapeseed protein isolate is reported to be

between 6000 and 8000 dollar per ton, equivalent to 5.22-6.96 euro per kilogram [19]. The *TOR* process is therefore not economically feasible when average market values are considered. The cost price of the protein isolates produced in the *BUR* process is well below the cost price range of the *TOR* process and *DAP protein concentrate.* The *BUR* process is expected to be feasible when average market values for the produced rapeseed protein isolates can be achieved.

The cost price range of the *DAP protein concentrate* is between 9.55-18.59 euro per kilogram, when the side-products are considered to have no market value. This cost price is high, given that commercial rapeseed concentrate has a market value of around 1-1.50 euro per kilogram. However, valorisation of the side-products in the *DAP* process contributes to the market value. For this reason, the cost price of the protein concentrate including the oil body cream and pellet side-products was accounted. The cost price including these products is much lower (0.47-0.91 euro per kilogram, see table 22).

To evaluate the economic feasibility the weighted average market value of the total products (protein concentrate and side-products) was determined. The weighted average market value was calculated as shown in table 23, based on the estimated market value of each individual product. The market value of each product was estimated based on a low and a high comparable product market price.

The low market value of the protein concentrate was based on commercial rapeseed protein concentrate (1.50 euro per kilogram). Whey protein concentrate was chosen as price basis for the higher market value, assuming that the produced protein concentrate has more valuable properties. Whey protein concentrate (80% protein) was reported to have a market value of 11 dollar per kilogram, equivalent to 9.57 euro per kilogram [19].

The oil body cream is a new product and has no comparable market value. When the oil body cream is evaluated as oil, the market value would be around 0.66 euro per kilogram [48]. However, because this product has potential emulsifying properties the minimum value was estimated at 1.50 euro per kilogram, equal to commercial rapeseed concentrate. Intact rapeseed oil bodies can also be used in cosmetics industry with an estimated value of 30 dollar per kilogram. This market value was selected as high value price basis.

The pellet was valued based on commercial rapeseed meal commodity price. Because the pellet which is produced as side-product in the *DAP* process has a relatively high moisture content of 84%, the economic value of this product may be lower than that of commercial rapeseed meal. For that reason, the low market value was estimated at 50% of the rapeseed meal price.

TABLE 23: WEIGHTED AVERAGE MARKET VALUE ESTIMATION OF *DAP ALL PRODUCTS*

Product	Price basis	Minimum	Maximum
Protein concentrate (dry)	LOW: Commercial rapeseed protein concentrate HIGH: Whey protein isolate	€ 1,50	€ 9,57
Oil body cream (wet)	LOW: Commercial emulsifier HIGH: Oil body cream cosmetics	€ 1,50	€ 26,09
Pellet (wet)	LOW: Rapeseed meal minus 50%[1] HIGH: Rapeseed meal FOB Hamburg	€ 0,10	€ 0,20
Weighted average market value (euro per kg product)		**€ 0,75**	**€ 11,33**

[1]*Discount based on low protein content and high moisture content. Protein content pellet DAP process is 15.6 wt.% DM. Protein content commercial rapeseed meal is around 40 wt.% DM.*

To make the process economically feasible, the total weighted average market value should at least be 1.00 euro/kilogram product, based on the maximum cost price including a margin of 10%. The weighted average

market value based on a low price basis (0.75 euro/kilogram, table 23) is below this margin. Therefore, the process may not be economically feasible if low market value prices are achieved at high production costs.

High market value prices for the oil body cream and protein concentrate are necessary to justify the high processing cost caused by freeze-drying and the high water consumption for downstream purification. The real market value of the specific products of the *DAP* process has to be studied based on the special functional properties and market application. Furthermore, the cost price can be optimized if water consumption and energy consumption of the process are reduced.

5 CONCLUSIONS

This study shows that the aqueous extraction process developed within the TiFN project "Sustainable Ingredients" is promising in terms of total protein yield of the protein concentrate; 39%. Including the side products (oil body cream and pellet) the protein recovery is 68%. This is considerably higher than the benchmark processes of the University of Toronto (*TOR*) and Burcon NutraScience Corporation© (*BUR)* which have protein yields of 32% and 20% of total rapeseed protein, respectively.

Both the benchmark processes of *TOR* and *BUR* produce two protein isolates of high purity. The protein isolates produced in the *BUR* process have a protein content of 100% on a dry matter basis and the protein isolates produced in the *TOR* process contain 87% and 92% protein on dry matter. The designed aqueous process produces one protein concentrate containing both protein fractions. The resulting protein concentrate has a lower purity (67% on dry matter basis). However, techno-functional properties (such as emulsifying characteristics) of the protein product produced in the designed aqueous extraction process might be superior, because mild extraction conditions are applied together with the use of intact rapeseed as starting material. Also, less fractionation steps are applied, which makes the process more efficient compared to the benchmark.

The absence of the commercial oil extraction process in the *DAP* process results in a lower oil recovery relative to the benchmark processes; during commercial oil extraction oil recoveries of 99% of total rapeseed oil are achieved, whereas the oil recovery in the oil body cream produced in the designed aqueous extraction process amounts 67%. However, in the designed aqueous extraction process oil is recovered in the native form of oil bodies, which have promising emulsifying properties.

The overall energy consumption of the designed aqueous extraction process (1.74-2.04 MJ per kilogram rapeseed processed) is lower compared to the benchmark process of *BUR* (2.24-2.52 MJ per kilogram rapeseed processed). The freeze-drying operation is the biggest contributor to the total energy consumption of the designed aqueous process and requires around 82% of the total energy.

The water consumption of the designed aqueous extraction process (48 kilogram water per kilogram rapeseed processed) is high compared to the *BUR* benchmark process (7 kilogram water per kilogram rapeseed processed). Especially the downstream purification water consumption is high, resulting in high wastewater production that has a significant environmental impact.

The economic evaluation of the designed aqueous extraction process shows that if only the protein concentrate is valorised, the cost price ranges between 9.58 and 18.58 euro per kilogram. This cost price is too high to make the process economically feasible, given the low market value of commercial rapeseed protein concentrates (around 1.50 euro per kilogram). However, when also the oil body cream and pellet side-products are valorised, the cost price range is reduced to 0.47-0.91 euro per kilogram product. The weighted average market value of the protein concentrate and the side-products is estimated between 0.75-11.33 euro per kilogram product. The process is therefore not economically feasible if low market values are obtained at high manufacturing costs. However, if high product quality and value is created the process becomes economically viable.

6 RECOMMENDATIONS

Because yield, efficiencies and product quality of the designed aqueous extraction process have considerable impact on the feasibility the process, the laboratory experiment data have to be validated with additional verification experiments. Larger scale pilot experiments have to be performed, since the unaccounted losses of the laboratory experiment results used in the study are significant. Pilot scale experiments will demonstrate the proof of practice and will provide more detailed data on yield and process conditions. These proof of practice data are necessary for further scaling up the process, gaining more knowledge on the product quality and right sizing of the water and energy consumption. Larger scale pilot experiments with industrial equipment will also provide a better understanding of the functional properties of the products produced. The process conditions at laboratory scale hugely differ from industrial scale in terms of pressure, diffusion and heat kinetics and applied shear forces.

The high water consumption of the process should be reduced to improve process sustainability and cost-efficiency, for example by studying the effect of pre-concentration by ultrafiltration prior to diafiltration.

Spray drying at low temperatures instead of freeze-drying can be studied to further optimize the overall process energy requirement. The effect of lower drying temperatures on protein functional properties has to be studied to validate the impact on product quality. Spray drying furthermore has the potential to reduce the CAPEX costs.

To improve economic feasibility, the real market value of the specific products of the designed aqueous extraction process has to be studied based on the special functional properties, focusing on high-end market applications.

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APPENDIX A. INVESTMENT AND ENERGY CONSUMPTION OF UNIT OPERATIONS

Table 1 lists investment costs of industrial scale machines and corresponding capacities, which are used for the capital cost and energy calculations in the models.

TABLE 1: INVESTMENT AND ENERGY CONSUMPTION OF UNIT OPERATIONS

Unit operation	Heat/electricity	Specific energy consumption	Investment - capacity	Source
Dehulling	Electricity	3.2 Wh/kilogram	250k euro - 10 t/h	Ref. A.1
Extraction tank with stirrer	Electricity	0.018 Wh/kilogram 0.03 Wh/kilogram	47k euro - 10 m^3	Ref. A.2
Blending	Electricity	0.92 Wh/kilogram	10k euro - 40 t/h	Ref. A.3
Screw press	Electricity	0.2 Wh/kilogram	30k euro - 8 t/h	Ref. A.4
Centrifuge	Electricity	1.5 Wh/kilogram	82k euro - 5 t/h	Ref. A.5
Freeze-drying	Electricity	2500 Wh/kilogram	1.369k euro - 625 kilogram/h	Ref. A.6
Meal washing	Electricity	0.03 Wh/kilogram	18.2k euro - 75 t/h	Ref. A.7
Heating	Heat	167.2 kJ/kilogram	34k euro - 100 m2	Ref. A.8
Precipitation tank	-	-	114k euro - 500 m^3	Ref. A.9
PVP filtration	Electricity	0.0417 Wh/kilogram	18.2k euro - 150 t/h	Ref. A.10
Vacuum filtration	Electricity	0.0207 Wh/kilogram	239k euro - 36 t/h	Ref. A.11
Cooling	Electricity	34.8 Wh/kilogram	310 euro/Wh	Ref. A.12
Ultrafiltration	Electricity	See calculation form	62k euro - 75 t/h	Ref. A.13
Diafiltration	Electricity	See calculation form	62k euro - 75 t/h	Ref. A.14
Filtration	Electricity	0.0417 Wh/kilogram	37k euro - 75 t/h	Ref. A.15
Spray drying	Electricity Heat	48.3 Wh/kilogram 4,696 kJ/kilogram	600k euro - 961 kilogram/h	Ref. A.16

Dehulling

A.1 Dehuller

In the dehulling process seed hulls need to be detached from seed kernels. The company Bühler has developed a state of the art industrial dehulling machine [49]. In this machine, the seeds are supplied by a screw feeder to a revolving rotor. The rotating rotor then accelerates the seeds by centrifugal forces, causing them to clash against impact cones. The collosion with the impact cone results in deformation of the seeds and detachment of the hulls from the kernels (see figure 1).

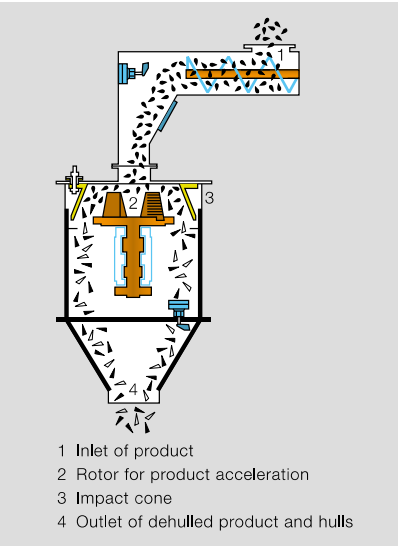

FIGURE 1: WORKING PRINCIPLE IMPACT DEHULLER OF BUHLER GROUP

The dehulling machine has a throughput of 180 tons of seeds per day, which equates to 7500 kilogram per hour. The main motor has a power requirement of 15-22 kW, whereas the feeder has a power requirement of 0.37 kW. Using this information, the specific energy consumption of the machine is calculated as follows:

$$Equation\ 1: \quad Specific\ energy\ consumption\ \left(\frac{Wh}{kg}\right) = \frac{Total\ power\ requirement\ (W)}{Throughput\ (\frac{kg}{h})}$$

This gives a specific energy consumption of 2.98 Wh per kilogram of seed processed.

Hull seperator

The dehulling results in a mixture of seed hulls and kernels. The next operation is to separate the hulls from the kernels. This can be done by an industrial hull seperator, which is also developed by the company Bühler [50]. The working principle of the hull separator is shown in figure 2. An oscillating sieve table separates the dehulled seeds from the not dehulled seeds, while a suction nozzle removes the lighter hull particles by aspiration air. Not dehulled seeds, which are separated off by the sieve table, are recycled back to the feed stream of the dehulling machine. The hulls are finally removed from the aspiration air by use of a cyclone.

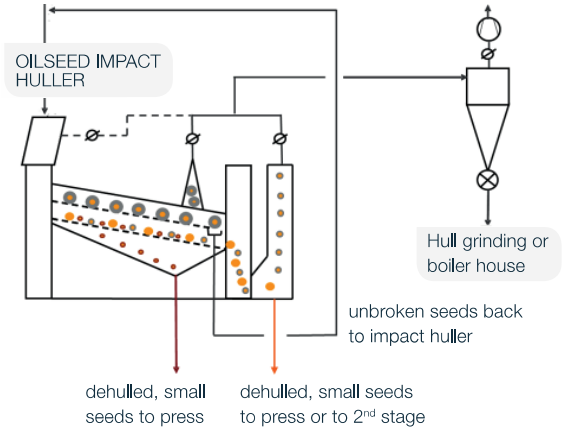

FIGURE 2: WORKING PRINCIPLE OF HULL SEPERATOR DEVELOPED BY BUHLER GROUP [50]

Unfortunately, the capacity of the hull separator for rapeseed hull-kernel separation is not provided. It is assumed that the capacity for rapeseed processing is comparable to the capacity for soybean processing. The GrainPlus 20 OL© type of hull separator has a capacity of 240 tons per day, which corresponds to 10,000 kilograms per hour. The oscillating sieving screen has a power requirement of 0.75 kW. The feeder roll, which serves for equally distributing the hull-kernel mixture on the sieving screen, also has a power requirement of 0.75 kW. Using equation 1, it is calculated that the specific energy consumption of the hull separator machine is 0.15 Wh per kilogram processed hull-kernel mixture.

The energy consumption of the aspiration fan must also be taken into consideration when estimating the energy requirement of the dehulling process. The hull separator has an aspiration connection of 15,000 m^3/hour. The power consumption of an aspiration fan corresponding to an aspiration rate around 15,000 m^3/hour, is estimated at 30 W per 1000 m^3/h, using the technical data on aspiration fans marketed by Vostermans Ventilation [51]. The power consumption for an aspiration rate of 15,000 m^3/hour is estimated at 450 W, using equation 2.

$$Equation\ 2: \quad Power\ consumption\ (W) = \frac{Aspiration\ rate\ (\frac{m^3}{h})}{1{,}000\ (\frac{m^3}{h})} * 30\ W = \frac{15{,}000}{1{,}000} * 30 = 450\ W$$

The aspiration connection of 15,000 m^3/hour corresponds to a throughput of 10,000 kilograms per hour in the hull separator. The specific energy consumption attributed to the aspiration is calculated to be 0.05 Wh per kilogram seed processed, using equation 1.

The total specific energy requirement of the dehulling process is the sum of the verified energy consumptions of the dehuller, the hull separator and the aspiration fan and corresponds to 3.2 Wh/kilogram seed processed. The total investment costs of the dehulling operation, including hull separation, are estimated at 250k euro for 10 tons per hour capacity.

A.2 Extraction tank with stirrer

For the extraction unit operation an extraction tank is used. The volume of the extraction tank depends on the designed extraction time, which determines the hydraulic retention time. The volume of the tank determines the stirring energy and the pumping energy required for filling the tank with extraction process water.

The stirring energy is calculated using the next formula [52]:

$$E_{stir}[\mathrm{J}] = \frac{N_P * \rho_{mix} * N^3 * d^5 * t}{\eta_{stir}}$$

$$E_{stir(1000\ l)} = \frac{0.79 * \rho_{mix} * 1.417^3\ \mathrm{s}^{-3} * 0.373^5\ \mathrm{m}^5 * t}{0.9} = 0.0180\ \mathrm{m}^5\mathrm{s}^{-3} * \rho_{mix} * t$$

Based on a 1,000-litre tank filled with water, the stirring energy according to this formula amounts 18 W.

The energy required for filling the extraction tank is based on the specific pumping power uptake derived from an industrial pump characteristic at a head of 5-meter water column (*see Appendix B*). The specific pumping energy is calculated at 0.03 kWh/m^3.

The investment costs of a stirred tank are based on the specified investment costs of a 10 m^3 tank equipped with a stirrer. The investment costs are 47k euro for a 10 m^3 tank [53].

A.3 Blending

Kitchen blender operations applied in laboratory processes are generally substituted by rotor-stator type homogenizers when the process is scaled-up to industrial operation [52]. In an industrial homogenizer, liquid and solids enter the machine at the inlet and are subsequently mixed and homogenized using the rotor-stator principle. The high shear forces generated in the rotor-stator generate the energy for homogenization. The intensity varies depending on the amount of shear forces applied. A typical example of an industrial homogenizer, the ULTRA-TURRAX UTL 1000 machine of supplier IKA©, is shown in figure 3 [54]. This homogenizer type generates moderate shear forces, which is probably suiting the goal of the blending process operation in the protein extraction process. The blending operation should not generate too much heat, as a too high heat input could have an impact on the protein functionality. The selected UTL1000/30 model has a capacity of 40,000 litres per hour and a power requirement of 36.8 kW [54]. Intensive homogenizers generally have higher power consumptions.

FIGURE 3: INDUSTRIAL BLENDER DEVELOPED BY IKA [54]

The process liquid that is blended is aqueous (consisting mostly of water); it is therefore that the capacity of the machine corresponds to 40,000 kilogram of process solution per hour. Using equation 1, the specific energy consumption of the homogenizing operation is verified at 0.92 Wh/kilogram solution processed.

The investment cost for the 36.8 kW blender is estimated at 10k euro.

A.4 Screw press

A screw press is a machine that used in various industries for dewatering and separation of liquids from solids. Screw presses are machines in which continuous separation of liquids from solids is based on mechanical forces. The mechanical forces are generated by a slowly rotating screw, which compresses the incoming solids. Compression of the solid material is accomplished by configuration of flights on the screw. Compression is also achieved by the increasing shaft diameter of the screw towards the solids discharge end. Besides, a pressure cone is installed at the solids discharge end. The pressure cone basically can be regarded as an opening through which the solids must pass, that is kept shut by air pressure. As a result of the gradually increasing pressure, liquid is being squeezed out. The screw press is encompassed by a tubular screen, through which the squeezed liquid is discharged [55].

The energy consumption of a screw press depends on the dry matter content of the feed as well as on the degree of dewatering. When a screw press is applied in manure dewatering, in which the manure feed stream has a high dry matter content, the specific energy consumption is relatively high (about 1,5-2 kWh/ton) [56]. Screw presses are also frequently applied in dewatering of sludge from wastewater plants. The Screw Press 40, marketed by the company Alfa Laval, is designed for dewatering of sludge without former sludge thickening. The machine has a power requirement of 2.2 kW and a throughput of 10 m^3/h and 20 m^3/h for digested sludge and waste activated sludge, respectively. This corresponds to specific energy consumptions of around 0.11 - 0.22 kWh/m^3 for sludge dewatering.

For the screw press operation in the aqueous extraction process, the specific energy consumption is estimated at 0.2 kWh/ton. The energy consumption is assumed to be comparable to dewatering of sludge without former thickening, as wastewater sludge is also aqueous.

The investment costs of a machine with a capacity of 8 tons per hour are reported at 30k euro [56].

A.5 Centrifugation

For industrial concentration of protein containing process water a decanter centrifuge is applied frequently. The working principle of a three-phase decanter centrifuge is shown in figure 4 [57]. The bowl wall of the centrifuge rotates very quickly, creating centrifugal forces that deposit the solids on the wall. A scroll conveyer subsequently conveys the solids towards the solids discharge end. The resulting clarified liquid then flows towards the liquid discharge area, while centrifugal forces separate the liquid into two distinct phases: the aqueous layer and the oil layer. The separated aqueous phase and oil phase can be collected separately.

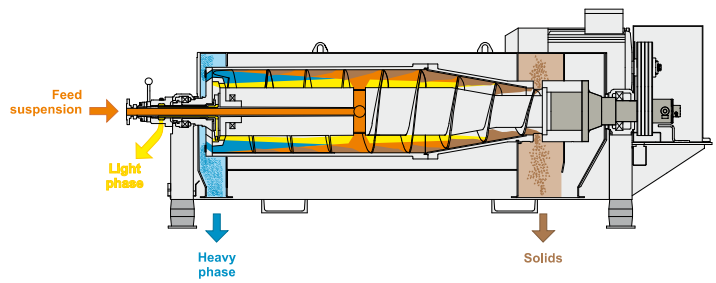

FIGURE 4: WORKING PRINCIPLE THREE-PHASE DECANTER CENTRIFUGE [57]

The energy consumption of a decanter centrifuge depends on various factors including the throughput, i.e. hydraulic residence time, and the rotational bowl speed. Both factors also determine the separation efficiency. A mid-range type two-phase decanter centrifuge has a hydraulic feed capacity of 3-50 m^3/h and power

consumption between 15-60 kW [58]. Assuming an average hydraulic throughput of 26.5 m^3/h and power requirement of 37.5 kW, the specific energy consumption is estimated at 1.4 kWh/m^3. The energy consumption of the centrifugation operation in the aqueous extraction process is estimated at 1.5 kWh/ton comparable to the described two-phase decanter. A three-phase decanter does have an extra outlet, which does not significantly consume additional energy.

The investment cost of an industrial scale decanter with a capacity of 5 tons per hour is reported at 82k euro [56].

A.6 Freeze-drying

Freeze-drying, also termed lyophilization, is a very gentle drying technique. In the freeze-drying process the material that must be dried is frozen, after which water is removed by sublimation. During sublimation, frozen water is directly transformed to water vapor, thereby skipping the usual melting phase. To achieve sublimation of water, vacuum is applied and energy in the form of heat is supplied. The water vapor that is produced in the sublimation process is condensed on ice condensers. Due to the low temperatures applied and absence of the liquid phase, freeze drying minimizes negative effects on protein functionality during the dehydration process [59]. A disadvantage is the large energy requirement of industrial vacuum freeze-drying operations.

The FD1500 type industrial scale freeze dryer, marketed by Cuddon Freezedry, has an evaporation capacity of 0.0625 ton per hour and a corresponding budget price of 1.369k euro (personal communication, May 14, 2018). The energy requirement of freeze-drying depends on the specific moisture extraction rate (SMER). Industrial vacuum freeze-drying operations generally have SMER values of 0.4 kilogram water/kWh, or even below [44]. A SMER value of 0.4 kilogram water/kWh is equivalent to an energy requirement of 2500 Wh/kilogram water condensed.

A.7 Meal washing

Meal washing is a unit operation where washing water is transported to a perforated filter belt, plate or screw. The required energy consumption for this unit operation mainly consists of pumping energy for transporting the process water. This energy consumption is derived from the pump characteristic of a 114 m^3 per hour centrifugal pump with a motor of 4 kWh. The working point of this pump is taken at a hydraulic head of 5 meters (*see Appendix B*). The specific energy consumption is calculated at 0.03 kWh/m^3.

The investment costs are based on an industrial scale designed filter with an effective surface area of 5 m^2. The required washing water velocity is set at 15 m/h, resulting in a capacity of 75 m^3/h. The investment cost of this filter unit operation are reported at 18.2k euro [53].

A.8 Heating

The required equipment for the heating step in the *TOR* process is designed on the target temperature difference to the inlet temperature of the process water, the flow rate and the temperature of the heating medium.

The solution has to be heated to 60 °C, starting at ambient temperature (20 °C). The specific required heating energy is calculated, assuming a specific heat similar to water:

$$\boldsymbol{Q = m * c * \Delta T \rightarrow \frac{Q}{m} = c * \Delta T}$$

$\frac{Q}{m}$ *= Heating energy per kilogram solution (kJ/kilogram)*

c *= Specific heat (kJ/kilogram*°C) = 4.18 kJ/kilogram*°C*

ΔT = *Target temperature difference (°C) = 40 °C*

This gives a required specific heating energy of 167.2 kJ/kilogram process solution. When a flow rate of 1 kilogram/h is processed, this corresponds to a required energy flow of 46.4 W.

The designed plate heat exchanger has a surface area of 100 m^2.

The following formula is used to calculate the duty of the heat exchanger:

$$\boldsymbol{Q = Kov * A * \Delta Tln}$$

Q = *Duty (W)*

Kov = *Overall heat transfer coefficient = 4500 W/m^2K* [60].

A = *Surface area heat exchanger = 100 m^2*

ΔTln = *Driving temperature difference (°C)*

The designed plate heat exchanger is operated in counter-current flow. The driving temperature difference is calculated with the next formula, assuming steam of 150 °C is used as heating medium.

$$\boldsymbol{\Delta Tln = \frac{(\Delta Tin - \Delta T\,out)}{ln(\frac{\Delta Tin}{\Delta Tout})} = \frac{(150 - 60) - (150 - 20)}{ln(\frac{150 - 60}{150 - 20})} = 108.8\ °C}$$

The 100 m^2 heat exchanger is calculated to have a heating duty of 48,960,000 W.

This heating duty is sufficient to heat 48,960,000 / 46.4 = 1,054,000 kilogram process solution per hour.

The investment cost of a 100 m^2 heat exchanger are reported at 34k euro [53].

A.9 IEP precipitation and PMM sedimentation

For designing the IEP precipitation and PMM sedimentation operations a sedimentation tank for the precipitation process is designed. The investment cost of an industrial scale 500 m^3 precipitation tank is reported at 114k euro [53].

A.10 PVP filtration

PVP filtration is applied on an industrial scale in the brewing industry for beer stabilization. Plate or candle filters are applied for separation of the added PVP adsorbent. Filtration velocities of 30 m/h are applied [61]. The pumping energy consumption is verified at 0.0417 kWh/m^3 based on a 4 kW pump with a hydraulic head of 10-meter water column (*see Appendix B*).

The investment costs of a 5 m^2 filter (resulting in a capacity of 150 m^3 per hour) are reported at 18.2k euro [53].

A.11 Vacuum filtration

Vacuum belt filters are commonly used for vacuum filtration on industrial scale. The solids are dehydrated on a filter belt, which is made of a weaved filter cloth. A vacuum pump removes the water from a cake layer of solids. The cake thickness is about 60 mm and the applied vacuum is between -100 mm Hg and -650 mm Hg. The belt speed is 1-50 m/min [62].

Based on design parameters of a 10 m^2 belt running at 2 m/min and a vacuum pump of 6.2 kW, the overall energy consumption is calculated at 0.0207 Wh/kilogram filtrate.

The investment costs of a 10 m^2 vacuum belt system with an estimated capacity of 36 tons cake discharge per hour, including vacuum pump, are reported at 239k euro [53].

A.12 Cooling

The energy required for cooling of process water as mentioned in the *BUR* process is calculated assuming a specific heat similar to water:

$$\boldsymbol{Q = m * c * \Delta T = \frac{Q}{m} = c * \Delta T}$$

$\frac{Q}{m}$ *= Heating energy per kilogram solution (kJ/kilogram)*

c *= Specific heat (kJ/kilogram*°C) = 4.18 kJ/kilogram*°C*

ΔT *= Target temperature difference (°C) = 6 °C*

This gives an energy requirement of 25.1 kJ/kilogram. This energy requirement is based on cooling process water from 10 °C to 4 °C with a heat exchanger which is connected to a refrigerator system.

The energy efficiency of a refrigerator system depends on the temperature difference between the cooling medium and the target temperature of the process water, which is defined as the cooling duty or amount of energy carried out by the refrigerator system. The system COP is defined as cooling duty (kW) divided by the electrical power input of the refrigerator system. For industrial refrigeration systems the COP value is on average 5 [63].

The required cooling energy can be calculated as follows, taking into account the system COP:

$$\boldsymbol{\frac{Q}{m} = (c * \Delta T) * 5 = 125.4 \frac{kJ}{kg} = 34.8 \frac{Wh}{kg}}$$

The investment costs of a cooling operation are reported at 310 euro/Wh [53].

A.13 Ultrafiltration

Ultrafiltration (UF) is a separation process based on a pressure driven membrane principle. The throughput or flux is depending on the pore size of the membrane and the composition of the process water. Ultrafiltration membranes usually have a pore size between 1 nm and 100 nm [64]. This pore size allows separation of compounds, which have a specific molecular weight. The membrane is characterized by the molecular weight cut off or MWCO value. This value varies between 1000 and 200,000 Dalton for UF membranes [64]. The separation efficiency of the membrane is determined by this value. For fractionating or concentration of substances like proteins the most optimum MWCO has to be selected based on the target retention molecular weight of the proteins which have to be retained.

An UF membrane can be operated in two operation modes: dead-end or cross flow. During dead-end operations all the transported materials accumulate on the membrane surface, whereas during cross flow filtration most of them are carried away in the retentate, which is fed back in the feed tank. Cross flow configuration is more suitable for treating water with high solids content and higher permeation fluxes can be achieved. For concentration of protein-containing process solutions, a cross flow operation is more suitable. Typical operating conditions for cross flow filtration are a cross flow velocity of 0.5 m/s at the membrane surface [65].

A typical process flow diagram of a cross flow operated UF filtration is shown in figure 5.

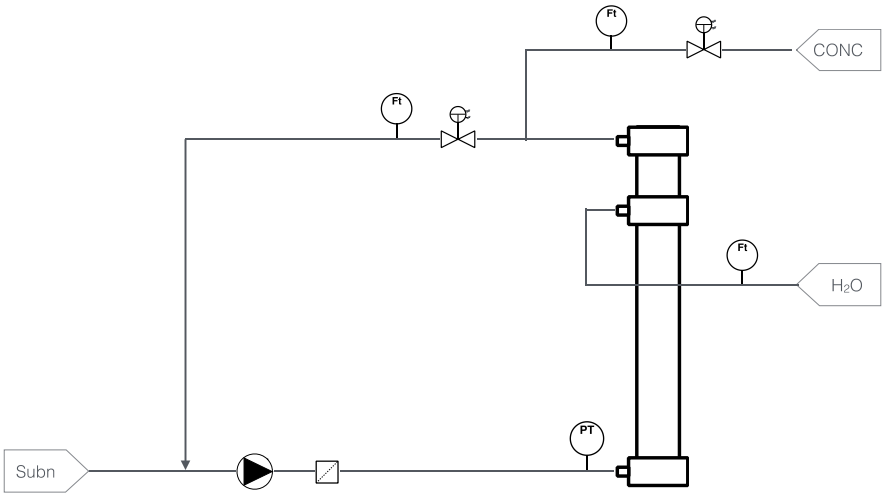

FIGURE 5: PROCESS FLOW DIAGRAM OF ULTRAFILTRATION UNIT

The trans membrane pressure (TMP) is controlled by controlling the pressure differential and the cross flow is maintained by the flow control valve, recirculating a part of the concentrate to the feed pump of the system. Backwash valves are installed for periodic backwashing and or forward flushing to remove the accumulated proteins on the membrane surface area.

The permeate velocity which passes through the membrane (flux) is driven by trans membrane pressure. The trans membrane pressure (TMP) is directly related to the flux:

$$Flux = \frac{permeate\ (liter/h)}{membrane\ surface\ area\ (m^2) * TMP\ (bar)}$$

The trans membrane pressure is defined by the pressure difference of the feed flow pressure and the permeate pressure. In most UF membranes the maximum TMP is limited to 1 bar [66].

The achieved flux also depends on the membrane pore size or MWCO (see table 2):

TABLE 2: CLEAN WATER FLUXES AT SPECIFIC MWCO VALUES

MWCO (kilo Dalton)	Clean water flux (L/m²*h*bar)	Source
100-150	120	[67]
10	60	[68]
5	15	[69]

The operational flux is also depending on the concentration of proteins in the process water. For calculating the energy consumption it is assumed that the flux drop-off due to concentration polarization at high protein levels is to a minimum of 20% of the clean water flux [70]. This value has to be validated in a pilot scale setup.

The ultrafiltration design characteristics have been modeled as shown in table 3.

TABLE 3: ULTRAFILTRATION DESIGN EXAMPLE

Membrane	10 kilo Dalton	
Protein concentration in	13	g/L
Protein concentration out	257	g/L
Membrane length	1,5	m
Membrane capillary diameter	0,9	mm
No. of capillaries	14.147	
Membrane surface area per module	60	m2
Capillary surface area	0,00900	m2
Cross flow velocity	0,5	m/s
Flux	38	l/m2.bar
TMP	0,8	bar
Permeate flow	1,8	m^3/h
Cross flow	16,2	m^3/h
Feed flow	18,1	m^3/h
Retentate flow	0,10	m^3/h
No. of membrane modules	3,5	
Membrane module cost	€ 1800,00	
Membrane lifetime	5,0	
Membrane replacement cost per year (disposables)	€ 1249,72	
Membrane replacement cost per kilogram rapeseed processed	€ 0,0004	
Capacity feed pump	63,0	m^3/h
Feed pump pressure	1,3	bar

The TMP was set at 0.8 bar. Based on a capillary diameter of 0.9 mm and a membrane length of 1.5 m, the surface area of one capillary can be calculated. The number of capillaries was calculated by dividing the total given membrane surface area by the surface area of one capillary membrane. The cross flow velocity was set at 0.5 m/s. The cross flow was calculated based on the capillary surface area. The flux range was set proportional to the average protein concentration conditions in the ultrafiltration concentration operation. The flux was maximized at the clean water flux of the specific membrane (see table 4). The number of required membrane modules was calculated by dividing the required flow rate by the permeate flow rate of one membrane at the set flux rate. The concentrate flow was calculated based on the membrane permeate recovery given in the model. The feed flow rate was calculated by summing up the calculated cross flow rate, permeate flow rate and concentrate flow rate. The pumping energy was subsequently calculated from the required total feed flow for maintaining pressure (set point 1,3 bar). The pumping energy was verified at 0.0556 kWh/m^3, based on 4 kW industrial scale pump at a hydraulic head of 13-meter water column (*see Appendix B*). The membrane cost and lifetime expectations were estimated based on information provided by a UF membrane system constructing company called Aramis.

TABLE 4: PROPORTIONAL RELATIONSHIP BETWEEN FLUX AND PROTEIN CONCENTRATION*

MWCO (kilo Dalton)	MIN FLUX ($L/m^2*h*bar$) protein concentration 300 g/L	MAX FLUX ($L/m^2*h*bar$) protein concentration 1 g/L	Proportional flux decrease per g/L (in protein concentration range 1-300 g/L)
10	12	60	0.16
5	3	15	0.04

**The maximum flux is based on the reported clean water flux and a protein concentration of 1 g/L*

**The minimum flux is estimated at 20% of the maximum flux and a protein concentration of 300 g/L*

A.14 Diafiltration

The diafiltration unit operation basically is a dilution process to remove small molecules, like salts, with the permeate using an ultrafiltration membrane. Impurities are removed by replacing them with a washing solution.

The design characteristics are similar to an ultrafiltration unit. The retentate of the ultrafiltration is continuously diluted with diafiltration buffer to lower the concentration of salts and impurities. The amount of washing water is defined in the model. It was designed that the volume before the diafiltration unit operation is the same as the volume after the operation, implying that the full washing water volume is permeated. The permeate contains the salts and impurities.

The membrane costs are based on the required membrane surface area, similar to ultrafiltration.

A.15 Filtration

To prevent clogging of the ultrafiltration membranes a pre-filtration step is necessary. A 10-micron candle filter is often used as a pre-filter. In some cases, this filter has an automatic cleaning device. The required pumping energy is based on a hydraulic head of 10-meter water column, which results in a specific electrical power consumption of 0.0417 kWh/m^3 (*see Appendix B*).

The investments cost for an industrial scale ultrafiltration pre-filtration unit with a capacity of 75 m^3 per hour are estimated at 36,695 euro, based on enquiry information provided by a company called Aramis.

A.16 Spray drying

Spray drying is commonly used in the dairy industry for drying e.g. whey or milk products. A spray-dryer operation consists of a tank where the product is sprayed with a nozzle in countercurrent with warm drying air. The small droplets entering the drying chamber evaporate the water in a few seconds. The moisture evaporation takes place in two stages. During the first stage, the temperature of the droplet is approximately equal to the wet-bulb temperature of the drying air, because the air is saturated by the evaporated water. If the air temperature is kept low, e.g. lower than 55 °C the wet bulb temperature of the droplet is less than 30 °C. This technique prevents heat damage of the resulting dried product. This stage lasts longer than the second phase. The second stage starts when there is no longer enough moisture to maintain saturated conditions at the droplet surface. Evaporation is then more difficult. During this stage the temperature rises, and the last remaining water is evaporated. Because the moisture content is low at this stage the risk of heat damage of e.g. proteins is limited [71].

The total energy consumption of an industrial spray-dryer is reported at 4.87 GJ/ton water evaporated, with a fuel to electricity ratio 1:27. This results in a specific electricity and heat consumption of 48.3 Wh/kilogram water evaporated and 4,696 kJ/kilogram water evaporated, respectively [45].

The investment costs of spray-dryer are reported at 600k euro for a spray-dryer with an evaporation capacity of 961 kilogram per hour [72].

APPENDIX B. SPECIFIC ENERGY CONSUMPTION CENTRIFUGAL PUMP

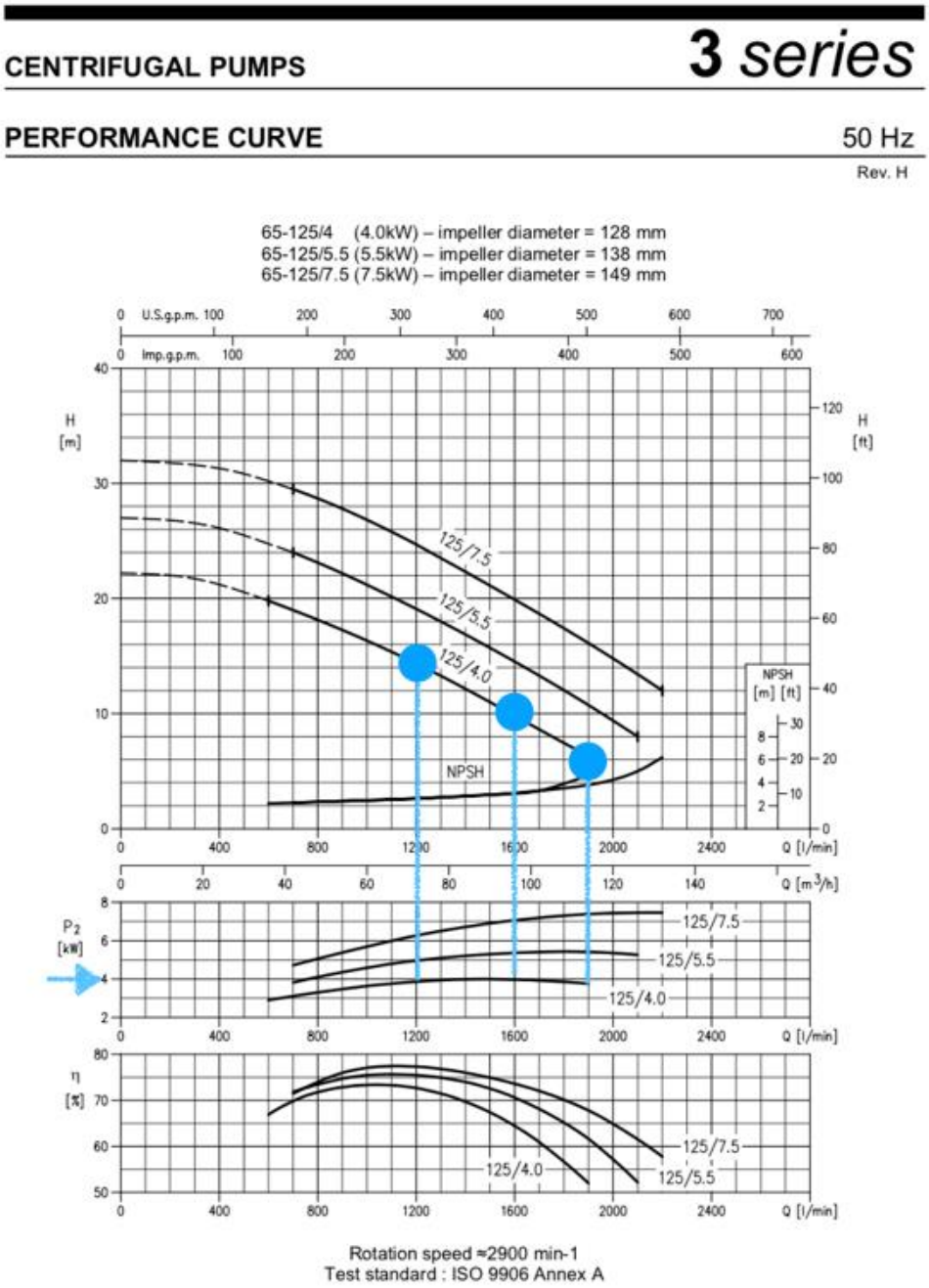

FIGURE 1: PEFORMANCE CURVE OF AN INDUSTRIAL CENTRIFUGAL PUMP (*SOURCE*: *EBARA PUMPS EUROPE S.P.A.)*

TABLE 1: CALCULATION OF SPECIFIC ENERGY CONSUMPTION OF AN INDUSTRIAL CENTRIFUGAL PUMP

	Hydraulic head 5 mWC	Hydraulic head 10 mWC	Hydraulic head 13 mWC
Power consumption (kW)	3.8	4.0	4.0
Flow rate (L/min)	1900	1600	1200
Specific electricity consumption (Wh/kilogram)	0.030	0.0417	0.0556

APPENDIX C. OPERATIONAL COST VALUES FOR COST PRICE CALCULATION

Table 1 lists operational cost values, which are used for the cost calculations in the models.

TABLE 1: OPEX COST VALUES (EURO PER UNIT)

	LOW	Medium	HIGH	Source
Rapeseed CIF Hamburg (euro/kilogram)	NA	0.37	NA	[53]
Meal FOB Hamburg (euro/kilogram)	NA	0.20	NA	[53]
Electricity (euro/MWh)	51.60	100.00	150.10	[81]
Natural gas (euro/MWh)	20.90	23.00	34.50	[81]
Water (euro/m^3)	1.00	1.50	2.00	[82]
Wastewater (euro/m^3)	0.80	1.00	2.00	[83][1]
Sodium hydroxide 50% m/m (euro/kilogram)	3.10	3.45	3.80	Alibaba
Hydrochloric acid 35% m/m (euro/kilogram)	2.70	3.00	3.30	Alibaba
Natrium sulfite (euro/kilogram)	3.00	3.35	3.70	Alibaba
PVP (euro/kilogram)	4.65	5.15	5.65	Alibaba
PVP disposal (euro/kilogram)	0.15	0.17	0.19	Alibaba
NaCl (euro/kilogram)	0.30	0.35	0.40	Alibaba

[1]An average pollution cost of the water authorities in the Netherlands amounts 50 euros per pollution equivalent (VE). The pollution equivalent is based on a daily discharge volume of 136 litres.
Formula: VE price 50 euro per year = 136 litres per day = 50 m^3 per year = 1 euro per m^3

APPENDIX D. ANALYSIS DATA DESIGNED AQUEOUS EXTRACTION PROCESS

TABLE 1: ANALYSIS DATA OF EXTRACTION PERFORMED ON 23-04-2018

	Mass wet (g)	Sample mass (g)	Moisture (%)	Dry matter (%)	Protein DM (%)	Oil DM (%)
Rapeseed	nd	nd	nd	nd	nd	nd
Hulls	nd	nd	nd	nd	nd	nd
Dehulled rapeseed	150	0	3.09	96.91	19.3	43.2
Liquid	1220.95	51.28	91.7	8.3	18.19	nd
Solids	80.46	14.31	56.43	43.57	13.9	31.7
Cream	110.16	38.46	55.9	44.1	7.86	81.8
Subnatant	915.52	44.89	97.3	2.7	40.39	nd
Pellet	101.34	37.45	84.4	15.6	15.64	38.2
Diafiltrate	nd	20	nd	nd	nd	nd
Wastewater	nd	nd	nd	nd	nd	nd
Freeze-dried	12.2	0	2	98	66.91	15.10
Condensate	nd	nd	nd	nd	nd	nd

TABLE 2: RECONSTRUCTED LABORATORY MASS BALANCE (INCLUDING SAMPLING LOSSES)

	IN					UNIT OPERATION	OUT					BALANCE DIFFERENCES				
	MASS (g)	MOISTURE (g)	PROTEIN (g)	OIL (g)	OTHERS (g)		MASS (g)	MOISTURE (g)	PROTEIN (g)	OIL (g)	OTHERS (g)	MASS (g)	MOISTURE (g)	PROTEIN (g)	OIL (g)	OTHERS (g)
Rapeseed	164.84	12.80	29.10	63.70	59.20	DEHULLING	150.00	4.60	28.00	62.80	54.50	0.04	0.00	0.00	0.00	0.00
Hulls							14.80	8.20	1.10	0.90	4.70					
Dehulled rapeseed + liquid	1350.00	1204.60	28.00	62.80	54.50	SOAKING BLENDING SCREW PRESS	1220.94	1119.60	18.43	50.00	32.90	48.56	39.57	4.69	1.68	2.50
Solids							80.50	45.43	4.88	11.12	19.10					
Liquid	1169.67	1072.59	17.66	47.90	31.52	CENTRIFUGE	915.52	890.80	9.98	1.94	12.79	42.65	34.68	1.38	0.18	6.41
Cream							110.16	61.58	3.82	39.74	5.02					
Pellet							101.34	85.53	2.47	6.04	7.30					
Subnatant	870.63	847.12	9.49	1.85	12.16	DIAFILTRATION	870.63	858.39	8.19	1.85	2.20	0.00	0.00	0.00	0.00	0.00
Diabuffer + wastewater	6096.85	6094.41	0.00	0.00	2.44		6096.85	6083.14	1.31	0.00	12.40					
Diafiltrate	850.63	838.67	8.00	1.81	2.15	FREEZE-DRY	12.20	0.24	8.00	1.81	2.15	0.00	0.00	0.00	0.00	0.00
Condensate							838.43	838.43	0.00	0.00	0.00					

**Values marked in red are assumed, because no analysis data was available (see table 1)*

TABLE 3: MASS LOSS PERCENTAGES CALCULATED FROM THE LABORATORY ANALYSIS DATA

UNIT OPERATION	MASS LOSS (%)	
DEHULLING	4.15	Hulls
SOAKING BLENDING SCREW PRESS	90.44	Liquid
	5.96	Solids
CENTRIFUGE	78.27	Subnatant
	9.42	Cream
	8.66	Pellet
DIAFILTRATION	87.50	Wastewater
FREEZE-DRY	98.57	Condensate

**Values marked in red are assumed, because no analysis data was available (see table 1)*