Tampere University of Applied Sciences

Purification of Proteins from Rapeseed Meal

Using Membrane Technology

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ABSTRACT

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Purification of Proteins from Rapeseed Meal – Using Membrane Technology

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The aim of this work was to study purification of proteins from rapeseed meal. Proteins were separated with membrane technology and studied with typical analyze methods like HPLC and with Kjeldahl method. This bachelor's thesis was done in the Université de Lorraine, in Nancy, France. Some of the experiences made were part of PhD thesis, so the exact results can't be shown.

Rapeseed is widely produced in EU countries (EU-28), Canada and China. It is widely used in EU countries, China and in the United States. Rapeseed meal is a by-product from the rapeseed crude oil production. It is primarily used for animal feeding. Because of the bitter off-taste of the extracts from the meal and because of the antinutritional factors such as glucosinolates, polyphenols and phytic acid, it is not used for human consumption. But there are now development projects to use some proteins from the meal for the human protein supply, e.g. in sausages.

After solid/liquid extraction of proteins from the meal, they are being purified with membrane technology. To find the best compromise between good quality protein isolates and cost efficiency production is the most difficult part, even today with all the technology and research information available. This difficulty has been on display at least 40 years. Decreasing the amount of phytic acid in the purification process and improving the yield of proteins were studied. With diafiltration is possible to decrease phytic acid content in protein isolate and with right pore sizes of the membrane the yield of proteins can be improved.

Keywords: rapeseed meal, protein, membrane technology

CONTENTS

GLOSSARY

α – helix	type of secondary structure in which the chain winds into a helix, with hydrogen bonds between residues separated by four positions in the sequence
ß – pleated sheet	another type of secondary structure, in which sections of main-chain interact by lateral hydrogen bonding
Ampholyte	a substance that can act as acid or basic
Chelate	to combine with a metal to form a chelate ring
Colloidal suspension	the term refers to a substance that has a solid permanently suspended in a liquid
Dalton (Da)	atomic mass unit, equal to 1/12 the mass of a single atom of the most abundant carbon isotope ^{12}C
Dispersion force	is the weakest intermolecular force
Dispersion medium	called also continuous phase or external phase, is the liquid, gaseous, or solid phase in a two-phase system in which the particles of the dispersed phase are distributed
GMO	genetically modified organism, an animal or plant whose genes have been changed scientifically
Hydrogen bonding	hydrogen bonds hold together proteins or other macromolecules, it is an interaction that involves a hydrogen atom
Nonpolar	consisting of molecules not having a dipole
Polypeptide chain	linear polymer of amino acids
Prosthetic group	constituting a nonprotein group of a conjugated protein
Proteases	any of numerous enzymes that hydrolyse proteins and are classified according to the most prominent functional group (such as serine or cysteine) at the active site
Supernatant	the usually clear liquid overlying material deposited by settling, precipitation, or centrifugation
Zwitterion	a dipolar ion

Sources used: merriam-webster.com; Lesk, 2004.

1 INTRODUCTION

The world population is estimated to grow rapidly for the next decades. All people need food to survive. Researchers are trying to solve the growth of a food shortage for human nutrition all over the world. In France researchers are trying to find right conditions to produce good quality protein isolates from plants in cost efficiency. It is more sustainable to produce plant proteins than animal proteins. The purpose is to be able to produce protein isolate which contains pure proteins and has needed functional properties. A novel source of plant protein was discovered from rapeseed meal.

Rapeseed meal is a by-product of a rapeseed oil production. Rapeseed is widely produced in EU-countries. Two biggest producers are France and Germany. Rapeseed doesn't flourish that well in Finland because of the short and relative cold summer. Turnip rape (Brassica rapa) is farmed in Finland instead, which needs different conditions than rapeseed. Rapeseed can only be farmed in few southern parts of Finland.

The work started by studying the subject diligently. The aim was to study the purification process of proteins from a rapeseed meal and to try find suitable conditions for protein isolate production. Everything begins with the solid/liquid extraction of rapeseed meal proteins. Then the extracted proteins will be purified with ultra- and diafiltration and in the end the proteins will be analysed with typical analyse methods like HPLC and Kjeldahl method. It is important to separate protein isolate that meets the requirements of food industry and has no extra colour, odour or flavour. Food industry wants different size proteins for different purposes. Those can be antioxidant properties, good digestibility and good techno functional properties, like foaming and emulsion capacities.

Antinutritional factors glucosinisolates and polyphenols weren't studied in this thesis. But both are secondary metabolites of plants. These water-soluble molecules extract with proteins. They can be separated with ultrafiltration from the proteins and studied afterwards. Glucosinolates exhibit antioxidant activities and antimicrobial properties but can also cause anaemia, hepatic and renal lesions if

used in quantity. Polyphenols offer protection against of development of cancer, osteoporosis, diabetes and cardiovascular diseases but in the other hand can cause kidney damage (tested with mice), tumour development and altered thyroid hormone production. (Ishida, Hara, Fukino, Kakizaki & Morimitsi, 2014, 48-49; Pandey & Rizvi, 2009, 270-278; Mennen, Walker, Bennetau-Pelissero & Scalbert, 2005, 326S-329S)

Third antinutritional factor, phytic acid, was under study with this thesis. Presence of phytic acid limits the purification of proteins. It can chelate with minerals and interact with proteins making minerals insoluble and prevent their absorption in digestive organs and decrease functionality of protein. Phytic acid has so small molecular weight that it is difficult to see it even with HPLC.

This thesis was done in research laboratory. In a laboratory like that the processes depends what is under study. Processes are not continuously like they would be in industry-based factories. Any of the results are not lined in anyway. Rapeseed meal was the source of proteins and even one analyses was done with sunflower proteins. Also, some of the quantities used in calculations are from another analytical method, analysis done by some other person. But it is normal to use available results in the laboratory.

2 BIOMOLECULES

The study of molecules and their reactions in living organisms is called biochemistry, it is based on the inorganic and organic chemical principles. Biochemistry gives the base and principles to understand the life sciences. There are small biomolecules that only have a few functional groups, and at the same time there are others that are enormous, and their biochemistry interaction is governed with large numbers of functional groups. (McMurry, Ballantine, Hoeger, Peterson, 2018, 589)

Breaking down food molecules, generating and storing energy, building up new biomolecules, and eliminating waste is continuously done by biochemical reactions. During these processes each biomolecule has its own place and role to play, regardless the enormous size and the complexity with interactions that some biomolecules have, there are no differences with functional groups and chemical reactions compared to the simpler organic molecules.
(McMurry et al., 2018, 589)

2.1 Proteins

An essential part of the diet is protein. They help to build, maintain and repair the body's structure. Human body can't store protein, so it is important to get enough of them every day from food. Protein can be found in many sources of food, including animals and plants. Proteins are broken down into amino acids in human body. Almost every metabolic process in the human body uses proteins or amino acids. (healthline.com; medicalnewstoday.com)

Amino acids can be classified to either essential or non-essential. There are around 20 types of amino acids human body needs to function correctly, and it can produce non-essential amino acids. There are nine of essential amino acids, that it can't produce so they need to be obtained from food. The 9 essential amino acids are presented in appendices 3 & 4. All the essential amino acids are needed for optimal health in the right ratios. (healthline.com; medicalnewstoday.com)

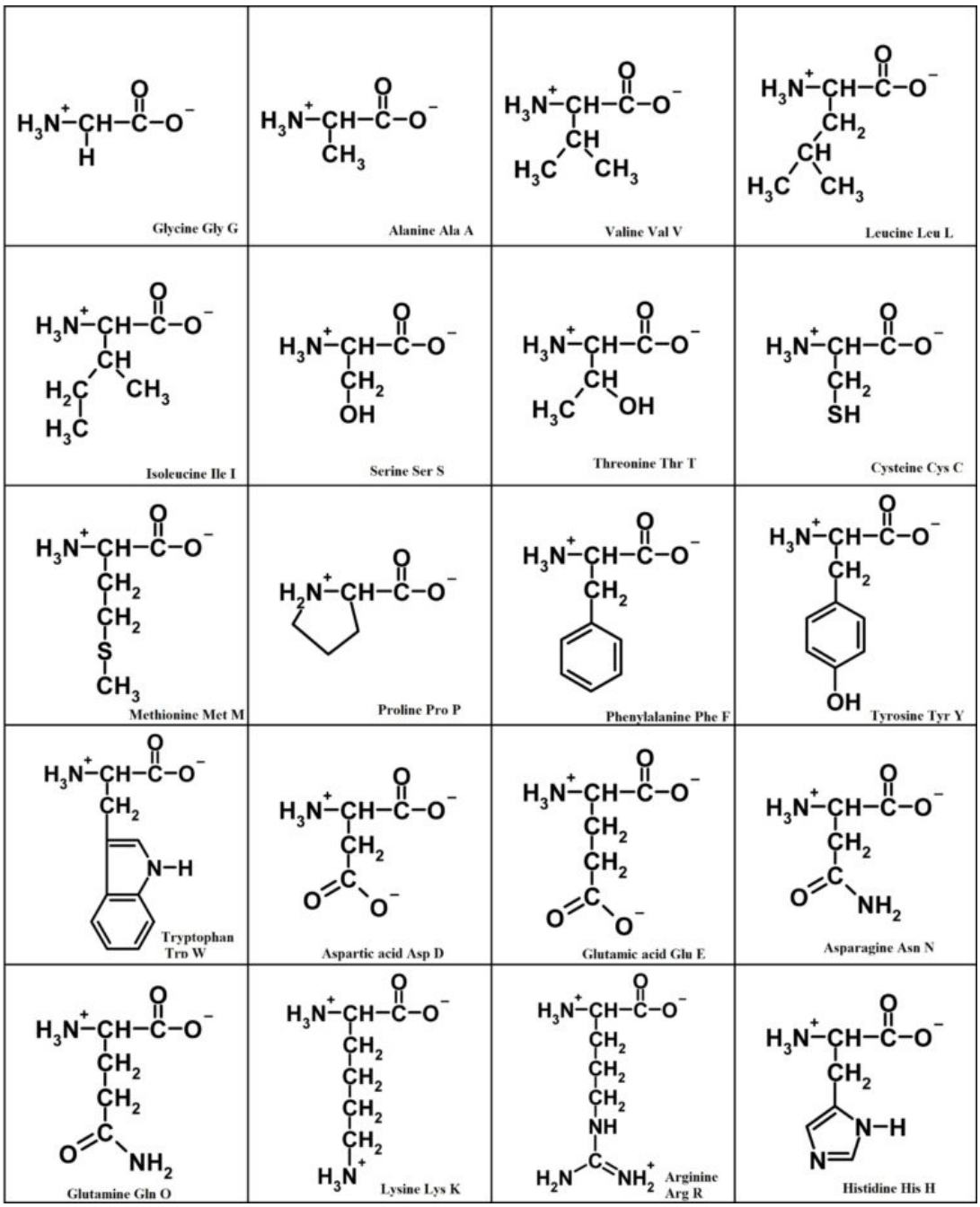
2.1.1 Structure of proteins

There are seven classes of proteins based on their functions. These seven classes are enzymes, structural proteins, signal proteins, contractile proteins, storage proteins, defensive proteins and transport proteins. Even though there are millions of proteins, all proteins are still made from the same building blocks, amino acids. In picture 1 the most common 20 amino acids are presented. More information of these amino acids, their structures and molecular weights, can be found in appendices 1 and 2. (Kent, 2000, 32)

When two amino acids combine by a condensation reaction a dipeptide is formed. The bond between these two amino acids is called peptide bond. More amino acids can be added to the dipeptide to compose a polypeptide chain. “Proteins consist of one or more polypeptide chains: they are polymers made up of amino acid monomers.” (Kent, 2000, 33)

Proteins have a unique three-dimensional shape which consists of one or more chains of amino acids (polypeptide chains). The shape of a protein is determined in four different structures: the primary structure, the secondary structure, the tertiary structure, and the quaternary structure. Previous level of structure determines always the next level. Simple proteins develop only of amino acids, conjugated proteins contain also a non-amino acid part which is called prosthetic group. (Kent, 2000, 34)

Proteins are big three-dimensional molecules. The order of amino acids is called the primary structure of the protein. The oxygen atoms in peptide groups has negative partial charge and the hydrogen atoms has positive partial charge. The different parts of protein molecule form hydrogen bonds and the molecule will coil to a spiral (Alpha-helix) or establish folds (Beta-pleated sheets). These distributions of helices and sheets are called secondary structure of protein which is held by hydrogen bonds. When protein molecules bind even bigger bundles a tertiary structure of protein is formed. Beside hydrogen bonds there are also ionic bonds between amino acid groups and dispersion force between nonpolar pendant groups. Many proteins have also quaternary structure, where more than one subunit join with different bonds. (peda.net; Lesk, 2004, 133)



PICTURE 1. The amino acids that make up proteins. (biochempages.com)

2.1.2 Plant or animal protein

It is two different things to extract proteins from plant or animal sources. That is because plant proteins are protected by a rigid cell wall that contains several interfering agents. Proteins, that are complex macromolecules, differs structurally and functionally from each other. Proteins are extremely sensitive to changes in their native environment. Changes in their native environment increase the risk

of degradation, denaturation and precipitation. Certain conditions must be considered to avoid this and to preserve their activity and integrity. (gbiosciences.com)

Protein extraction makes proteins unstable. When the cells and organelle members are dissociated, the solution exposes the proteins to different ionic concentration, pH and temperature. (gbiosciences.com)

To produce 1 kg of animal protein, feed is needed for 6 kg of plant protein. Livestock production systems spend fossil energy. Between plant and animal proteins the primary difference is their amino acid profile. Animal protein are more like to human proteins and they can be used more rapidly to human protein synthesizing reactions. Some plant proteins may take more time to digest and to use in a human body. Plant protein sources, seeds, nuts and beans, are incomplete providing the essential amino acids in suitable amounts depending from the source. Canola/rapeseed proteins have a balanced amino acid profile, providing all the nutritionally essential amino acids. (nutritionstudies.org; Pimentel & Pimentel, 2003, 660S-663S; Wanasundara, McIntosh, Perera, Withana-Gamage & Mitra, 2016, D407)

2.2 The source of proteins

The plant proteins in this study was purified from rapeseed meal. Rapeseed is commonly produced and used in France.

2.2.1 Rapeseed

Rapeseed, Brassica napus subspecies, is an oil crop in the Brassica family known as rape and oilseed rape, and ‘canola’ for a specific group of variety. Rapeseed can be related to broccoli, cabbage, cauliflower, mustard, and turnip. The plants of rapeseed grow from three to five feet tall and the flowers with four petals blossom in yellow. (agmrc.org)

Primarily the rapeseed is grown for its oil. For a profitable rapeseed production, a challenge is the limited use and markets of the meal remaining after oil processing. Rapeseed contains more than 40 percent oil. Over winter it will offer good soil cover to prevent soil erosion, it also yields large amounts of biomass, and suppresses weeds. Crops with GMO are widely grown across the world, but they are very questionable in Europe, some few strains are authorized to grow but countries like France have completely forbid their cultivation citing environmental risks. (agmrc.org; reuters.com)

2.2.2 Rapeseed meal

From the production of crude rapeseed oil comes a by-product which is called rapeseed meal. It is obtained after pressing process of the rape seed. Rapeseed meal is typically brown colour and has typical aroma without any inappropriate odours. There are some annual production rates in figure 1 of a rapeseed meal in Europe. Its production has more than doubled in the past twenty years. (enviengroup.eu)

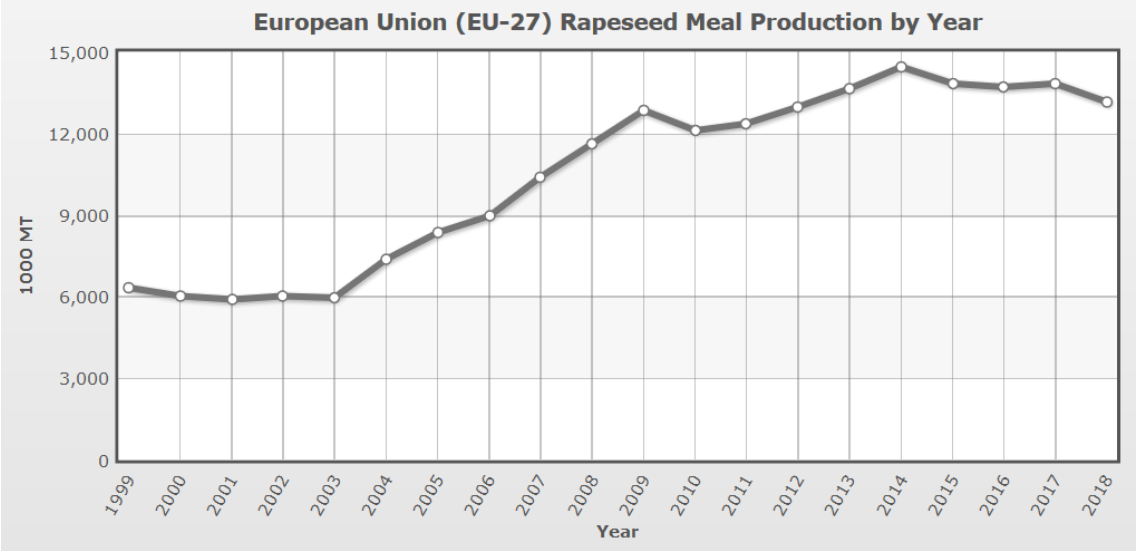


FIGURE 1. EU-27 rapeseed meal production. (indexmundi.com)

Globally 34 million tons of pressed rapeseed meal cakes come in to the world annually after crude oil production of a rapeseed. Rapeseed meal cakes contains 32-36 % nutritionally good quality proteins. Its marketing in EU-area as a novel food is allowed in 2014. (vtt.fi)

2.2.3 Usage of rapeseed meal

Rapeseed meal contains physiologically valuable protein, but it contains also up to 10 % secondary plant metabolites. High fiber content and residue of these antinutritional factors, phytic acid, glucosinolates and phenolic compounds, which causes bitter taste and dark colours in protein products, has limited its use in human nutrition, was written 2014 in Oilseeds and fats, crops and lipids, by Von der Haar, Müller, Bader-Mittermaier, and Eisner. In 2011 Wanasundara wrote in Critical Reviews in Food Science and Nutrition that utilization of rapeseed protein as human nutrition, because of its techno-functional properties such as foaming and emulsification capacity, is of growing interest. (Fezer, Herfellner, Stäbler, Menner & Eisner, 2018, 236-246)

Rapeseed meal can be used part of daily portion for milking and dry cows, replacement heifers, calves, growing and finishing cattle, suckler cows, ewes and rams along with hoggets and lambs. (kwalternativefeeds.co.uk)

The increasing need of protein for human nutrition and animal feeding leads the interest to new protein sources. Rapeseed meal was established as a raw material to producing protein isolate. Large amounts of press cakes and residues from production from edible oils and from oil extraction are available. The meal contains high-grade proteins comprised of essential amino acids. The resulting proteins can be used in food applications such as bakery products, ice creams, desserts, mayonnaise and sausages. Proteins can be hydrolysed to improve solubility in water as well as other properties. (foodnavigator.com; Von der Haar, Müller, Bader-Mittermaier & Eisner, 2014, D104; Vioque, Sánchez-Vioque, Clemente, Pedroche & Millán, 2000, 447-450)

Proteins in protein isolate are extracted and purified from the rapeseed meal. Protein isolate is very rich in proteins, it can even contain more than 90 % pure proteins. There is antinutritional factor called phytic acid that interacts with proteins and causes problems in the separation process.

There has been one major problem, bitter off-taste, using these protein isolates in human nutrition. Researchers in Technical University of Munich (TUM) has

found the substance that gives the unpalatable bitterness. The compound is called kaempferol 3-o-(2‴-o-sinapoyl-β-sophoroside) and it was found using mass spectrometric analyses and taste tests. Researchers believe that knowing the source of bitter off-taste gives better opportunity to develop technological processes producing protein-rich foodstuffs from rapeseed. In 2016 Nutra Ingredients reported that protein from rapeseed meal contains bioactive peptides and essential amino acids cysteine and methionine more than comparing to pea or soy protein. (nutraingredients.com)

2.2.4 Limiting factors for purification and use – Phytic acid

Phytic acid (IP6) is a natural antioxidant, that exists in cereals, natural oils, nuts and vegetables. It was considered an antinutritional factor for a long time. Because of its ability to chelate with minerals like calcium, copper, iron and zinc, which prevents absorption in the gastrointestinal tract. However, several studies have demonstrated its utility to prevent several pathological conditions and cancer. (Silva & Bracarense, 2016, 1357-62)

“The pH of the acidity or alkalinity of the dispersion medium of a colloidal suspension or an ampholyte at which the solute does not move in an electrophoretic field.” Abbreviation of the term isoelectric point is pI. (accessscience.com)

Phytic acid presence in food rises concerns because there is evidence its decreasing factor on bioavailability of many essential minerals. Phytic acid interacts with multivalent cations and/or proteins and forms complexes that might be insoluble or else unavailable under physiologic conditions. (Cheryan, 1980, 297)

The content of phytic acid has always impact of the cultivar, climatic conditions and year. The interaction between protein and phytic acid is highly dependent on pH. With very low pH, below proteins’ isoelectric point, the last amino, arginyl, histydyl or lysyl groups can be charged positively. A negatively charged phytate anion can form directly a complex with any of those mentioned groups. One phytate anion can interplay with two charged groups of protein, if there are satisfactory steric conditions. Depending on conformational conditions and the number of

positively charged groups, the protein molecule can naturally bind more numerous phytate anions at the same time. The arginyl and lysyl are the only groups positively charged at intermediate pH values, so with these conditions there exists a little possibility of electrostatic interactions. Interaction between protein and phytic acid is diminished if the pH is extremely high.
(Hídvégi & Lásztity, 2002, 59-64)

To improve protein nutritional value from rapeseed meal and to reduce phytic acid content a pre-treatment with phytase was tested. Phytase (EC 3.1.3.26) is a phosphoric monoester hydrolases, which can hydrolase phytic acid to yield inorganic orthophosphate, free moy-inositol and several lower esters of moy-inositol. This enzyme is distributed widely in plants, animal tissues, bacteria species and many fungi. In rapeseed meal cake the degradation of inositol phosphate was promoted by phytase addition. (Rodrigues, Carvalho & Rocha, 2017, 2641-2646)

Rodrigues, Coelho and Carvalho stated 2012 in Journal of Food Engineering that protein-phytate interaction depends on pH level and content of divalent ions. When proteins are positively charged, whereas phytate has a negative charge, which happens in acidic pH, a phytate-protein complex formation will be originate. When protein and phytate are both charged negatively, in alkaline pH, preferable conditions to reduce the phytic acid content of protein isolates is reached. After pre-treatment with phytase, the rapeseed meal decreased about 25 % of the amount of phytic acid. (Rodrigues et al., 2017, 2641-2646)

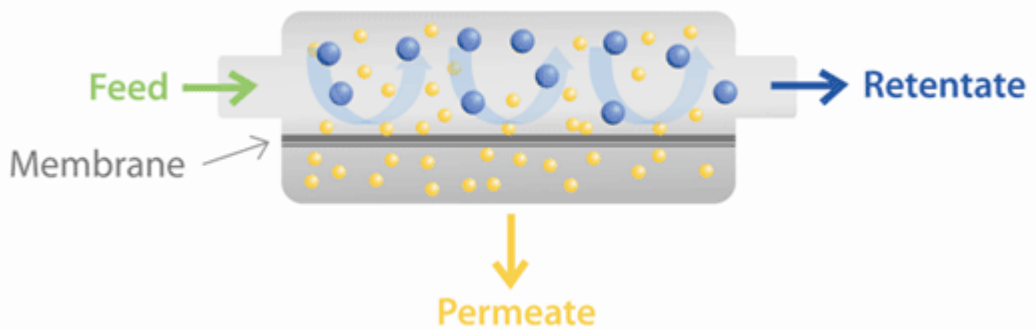
3 MEMBRANE TECHNOLOGY

In the membrane separation processes, the membrane acts as a selective barrier allowing free passage of one component while retaining another. Membranes are used widely in food industry, in the manufacture of dairy products. Automotive industry uses membranes for the recovery of electropainting baths. Water supply is made possible by the membranes for millions of people in the world. Large number of people suffering from kidney disease is cared and survived because of membranes. In the field of chemical industry membranes can be used e.g. purifying process gases. (Nunes & Peinemann, 2006, 3)

3.1 Four different methods

Two educational YouTube videos was found, where Distinguished Professor R. Paul Singh from the department of Biological and Agricultural Engineering from University of California, Davis, introduced basic terms and methods in membrane separation process. In next paragraphs are few of his thoughts.

Some terms which are frequently used when working with membranes are feed, permeate, retentate, flux and membrane fouling. Few terms are drawn in the picture 2 and the rest of the terms are just introduced.

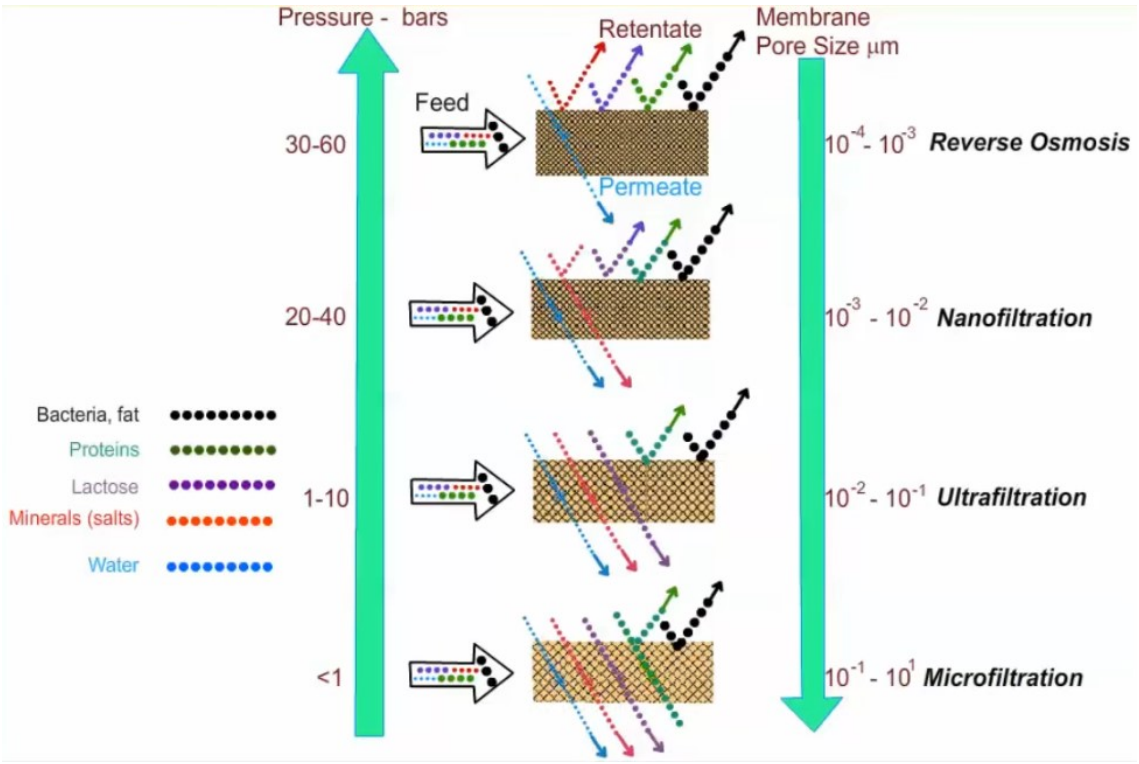


PICTURE 2. Terms used in membrane technology. (novasep.com)

Feed is the solution which is supposed to be concentrated or contain the molecule to be purified. Permeate is the filtrate, the liquid which will pass the membrane.

Retentate is the retained liquid. Flux is the rate of extraction of permeate expressed and its unit is litres per square meter of membrane area per hour (l/m^2/h). Membrane fouling means the solids deposited on the membrane surface. (Singh, 2016)

Membrane technology has four different methods of separating particles. They are called microfiltration, ultrafiltration, nanofiltration and reverse osmosis. Pressure used, and pore size of the membrane are the crucial factors which divides the process method. There are few examples of pressures and pore sizes that are used in these four different methods, and what kind of particles passes through in picture 3. (Singh, 2016)



PICTURE 3. Membrane separation performance. (Singh, 2016)

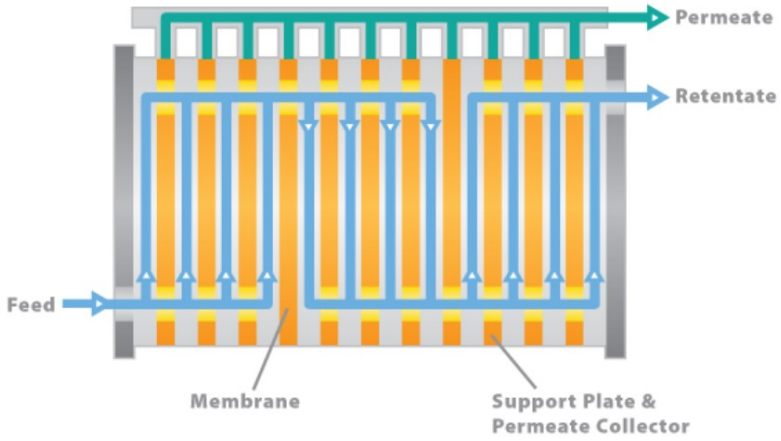
In membrane separation process, the membrane is the filter. Through the filter a liquid, under pressure, is forced to flow along the surface of the membrane. The flow sweeps the retained particles and the cake layer on the membrane surface is relatively thin. Therefor the resistance to filtration remains low, and the flux can be maintained high over long periods of time. (Singh, 2016)

3.2 Membrane models

There are at least four or five different membrane types, depending if the tubular membrane is made from polymers or ceramics.

3.2.1 Plate & frame membranes

Plate and frame membrane systems utilize membranes that have a plate like structure, what is then held together with a frame like support. Plate and frame membrane systems look very similar than plate heat exchanger. There are two different types of these plate and frame membrane systems: dead-end and cross flow. Cross flow filtration is also known as tangential flow filtration and there is more information on that in chapter 3.5.2. In picture 4 is a model configuration of plate and frame membrane system. (synderfiltration.com (a))

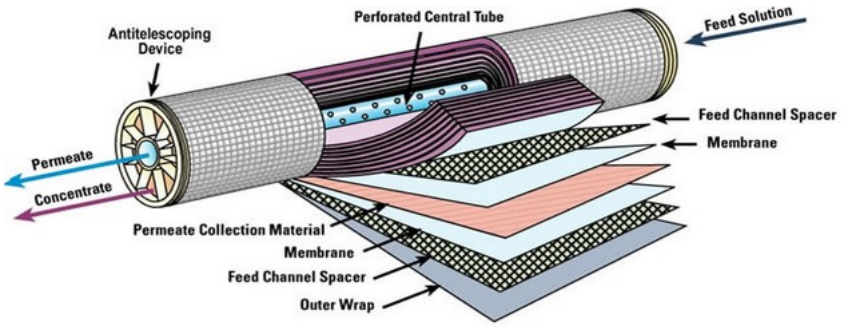


PICTURE 4. Plate and frame model membrane system. (synderfiltration.com (a))

Some advantages with this model are that plates can be easily removed or replaced. This system can be rotated if needed. And this system doesn't need any feed spacers. It has also low hold up volume. Low packing density is one of the biggest disadvantages with this system. It is also low in efficiency compared to the other models and it has large pressure drop. (synderfiltration.com (a))

3.2.2 Spiral wound membranes

There are feed spacers, permeate spacers and a permeate tube in these spiral-wound elements. In this model the feed travels through the flow channels for the length of the entire element. The feed gets concentrated in the end of the element body. A structure of a spiral wound membrane can be seen in picture 5. (synderfiltration.com (b))

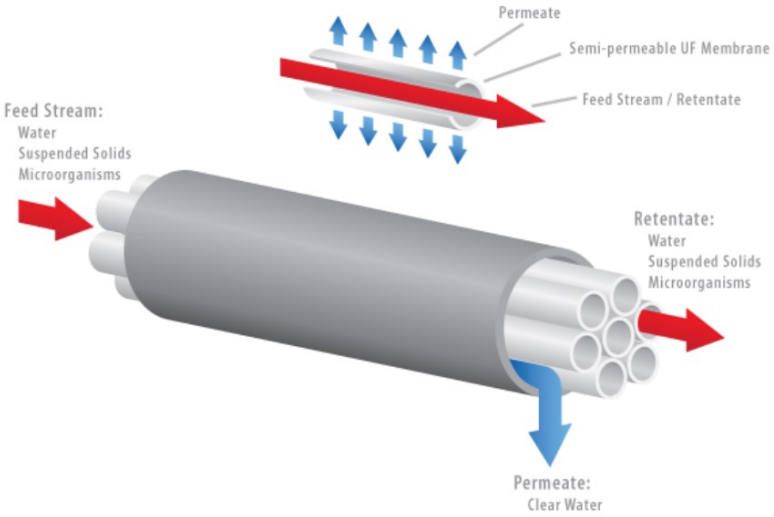


PICTURE 5. Spiral wound membrane structure. (lixus.net.cn)

Advantages with this module are that they can be made with many different spacers, membrane types, lengths and diameters. Typically, spiral wound elements dispense the best value in relation to membrane area and it is compact. Some disadvantages would be that spiral wound membrane elements can't be mechanically cleaned like tubular membranes. The packing density is lower than compared to hollow-fibre membranes. (synderfiltration.com (b))

3.2.3 Tubular membranes

Tubes that have outer diameter as half of an inch or an inch are typically packed in to a long pipe which have a PVC housing but can have a base either from polymer or ceramic. There is a minimum of two tubes, the inner membrane tube and the outer shell tube. Model of a configuration of tubular membrane can be seen in picture 6. (synderfiltration.com (c))

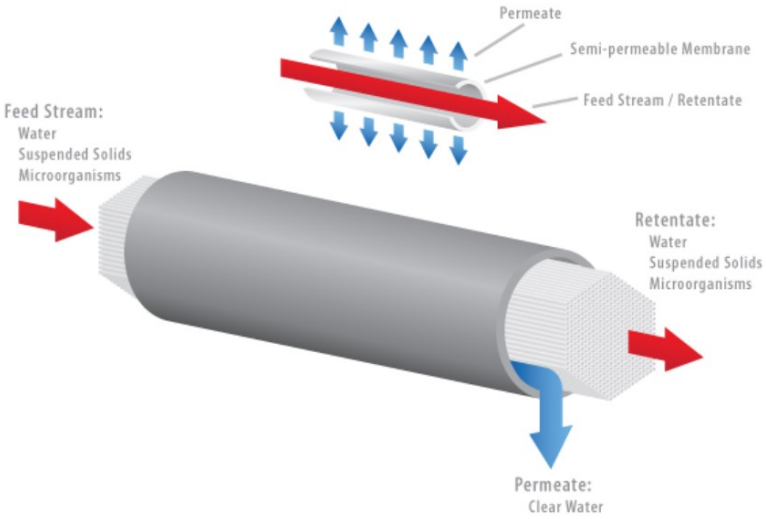


PICTURE 6. Tubular membrane structure. (synderfiltration.com (c))

As advantages, these systems admit robust cleanout methods, such as back-wash, harsh chemicals, and mechanical cleaning. Disadvantages would be very high start-up and operating costs, also high energy consumption and high hold up volume. (synderfiltration.com (c))

3.2.4 Hollow-fibre membranes

There are thousands of porous filaments, diameter ranging from 1-3,5 mm which are potted in a PVC shell in these hollow fibre membrane models. This model works on a same principle as tubular model. In picture 7 is a configuration of a hollow-fibre membrane. (synderfiltration.com (d))



PICTURE 7. Hollow-fibre membrane principle. (synderfiltration.com (d))

Because the strands are flexible, and the packing density is very high this model can achieve some filtration that cannot be achieved with other filtration models. This is the advantage of this system. It has also low hold up volume and low energy consumption. The disadvantages are breakage of fibres causes to change the whole unit and irreversible fouling. (synderfiltration.com (d))

3.3 Materials used in membranes

Many of the industrial membrane filters are made of natural or synthetic polymers, both are being called organic membranes. Natural polymers include cellulose, rubber and wool. Synthetic polymers include cellulose acetate (CA), Polyacrylonitrile (PAN), polyethylene (PE), polyethersulfone (PES), polyimide (PI), polytetrafluoroethylene (PTFE), polypropylene (PP) and polysulfone (PS). Advantages with these are high permeate flows and inexpensive price. With synthetic polymers advantages are wide tolerance of pH and temperature.
(asahi-kasei.co.jp; separationprocesses.com)

Membranes can also be called inorganic membranes, which are usually made from ceramics or metals. Some examples of used materials aluminium oxide (Al_2O_3) and zirconium oxide (ZrO_2), materials that have been tested as well are stainless steel (SUS) and glass (Sirasu Porous Glass, SPG). Advantages with these materials are chemical and heat resistance along with mechanical strength. Disadvantage is very high price. (asahi-kasei.co.jp; separationprocesses.com)

3.4 Molecular weight cut-of

Molecular Weight Cutoff (MWCO) is the retention properties of ultrafiltration membranes. A dilute globular solute (e.g. protein) which is retained 90 % of the membrane by the estimated molecular weight (MW) is referred to this value. Nevertheless, a membrane retention can have a direct effect because of a molecule’s shape. (pall.com)

After the sample volume is determined, selection of the suitable MWCO is the next step. The ability to retain more than 90 % of a solute which molecular weight is known in kilodaltons gives nominal ratings to MWCO values. It is recommended for proteins to select MWCO that are 3 to 6 times smaller than the molecular weight of the solute being retained. (pall.fi)

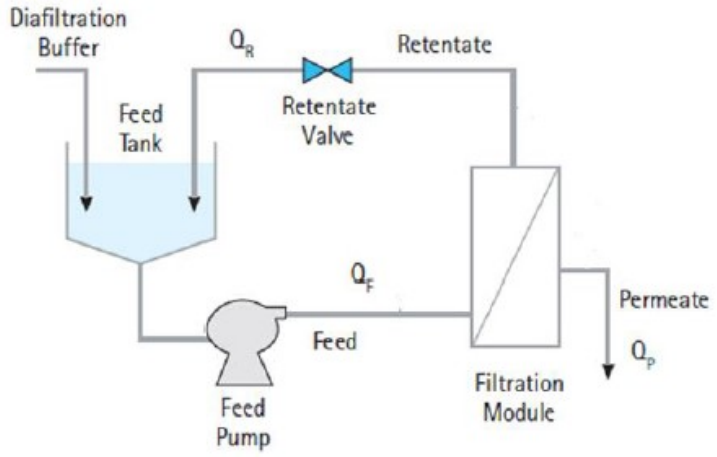
If the retention rate is under consideration it is preferable to choose three times lower membrane with MWCO. If the main concern is retention it is advisable to choose six times tighter membrane. It is important to recognize that molecular weight is only a general indicator and retention of a molecule by a UF membrane is completed by a variety of factors. It is important to consider also electrical charge, sample composition, sample concentration, molecular shape, and operating conditions. (pall.fi)

3.5 Ultrafiltration

UF (Ultrafiltration) is one of the membrane filtration method, which enables separation of solid matters and high molecular weight dissolved solids from the water. Typically, ultrafiltration is used to reclaiming proteins and preliminary purification for reverse osmosis method. (gronmark.fi)

3.5.1 Diafiltration

A specialized type of ultrafiltration process is called a diafiltration, where the retentate is diluted with water and re-ultrafiltered. It is done to reduce soluble permeate component concentration and to increase more retained component concentration. In picture 8 is a principle of a diafiltration. (uoguelph.ca)



PICTURE 8. Principle of diafiltration. (emdmillipore.com)

QF = feed flow
QP = permeate flow
QR = retentate flow

Diavolume (DV) is calculated as: DV = total buffer volume introduced to the operation during diafiltration / retentate volume. (emdmillipore.com)

3.5.2 Tangential Flow Filtration

Tangential flow filtration TFF, also known as cross flow filtration CFF, is a filtration technique where the surface of the filter is passed tangentially by the starting solution. Smaller components that the pores of the filter (membrane) are driven through the filter by pressure difference. Larger components than the filter pores, passes along the membrane surface and retain, flowing back to the feed container. TFF is simple in concept but needs detailed knowledge in proper execution and good filtration technique. In picture 9 is the TFF equipment used in laboratory. (processdevelopmentforum.com)

PICTURE 9. Tangential Flow Filtration System. (merckmillipore.com)

TFF differs in three ways from normal flow filtration, where the starting material simply passes through the filter. TFF use membrane filters exclusively, when conventional filtration may use membranes, glass fibre, paper, or other materials to separate components from the feed stream. TFF recirculates of the retentate solution, normal flow filtration passes the solution usually only once. TFF keeps the retentate as a solution and it may be recovered directly. Retentate recovery is uncommon in normal flow filtration and requires resuspension of the collected material in the filter. (processdevelopmentforum.com)

3.6 Transmembrane pressure

To maximize the flux, it is essential to choose suitable combination of feed flow and TMP. TMP (transmembrane pressure) is the average of pressure difference between feed and permeate stream. Correct combination of these two will minimize time and/or area of the membrane, allowing for optimal pump sizing and membrane area. In figure 2 is curve of TMP and filtrate flux. (merckmillipore.com)

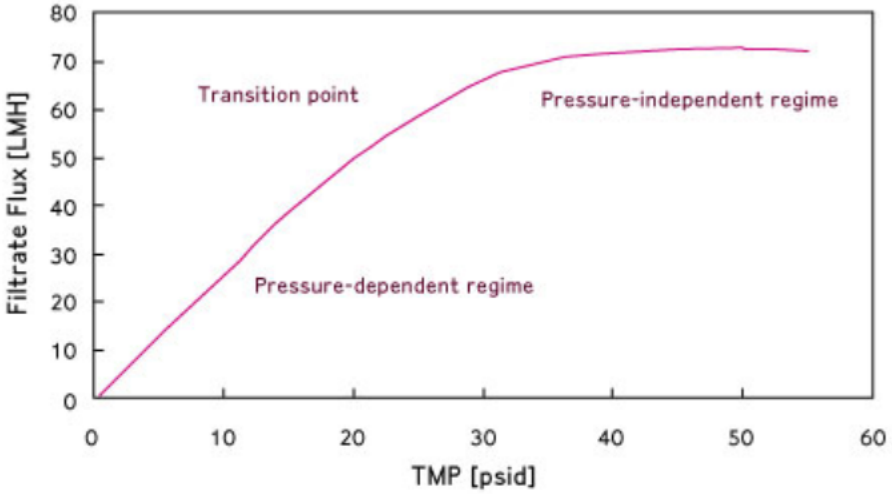


FIGURE 2. The curve of TMP and filtrate flux. (merckmillipore.com)

For standard UF/DF process, the knee of the curve shows the optimum TMP to running a process. In this point the highest flux will be achieved without excessive actuation pressure or fouling or reaching an excess high protein wall concentration. (merckmillipore.com)

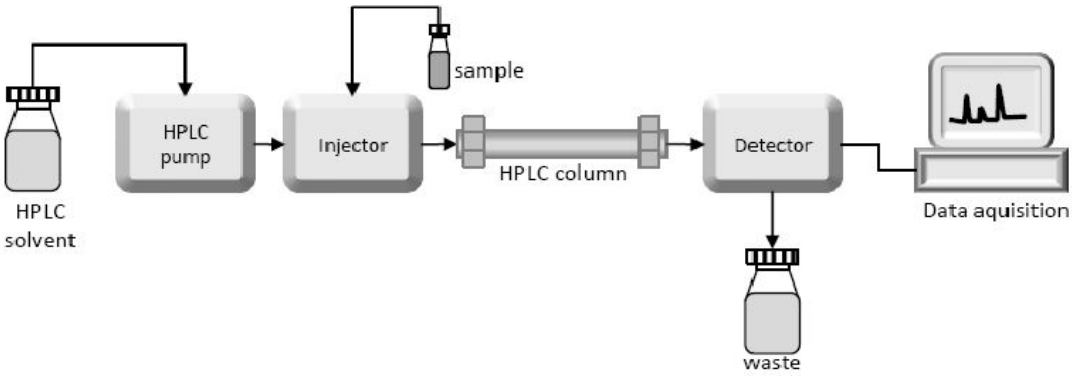
4 ANALYTICAL METHODS

After the proteins were extracted and purified with membrane filtration, they were analysed and calculated with following methods. These methods were also used to monitoring the process.

4.1 HPLC - High Performance Liquid Chromatography

A technique that separates mixtures of substances based on their molecular composition and structure is called chromatography. There is a stationary phase and a mobile phase. The stationary phase is usually a solid, or sometimes a liquid supported on a solid. The mobile phase is a liquid or a gas. The components of the mixture will be carried out with the flowing mobile phase through the stationary phase. Sample components that have weaker interactions with the stationary phase will move faster through the column than components with stronger interactions. The solvent is forced through a column under high pressures of up to 400 atmospheres, which makes it a lot faster, than just with the drip under gravity. All chromatographic separations, among other like HPLC, operate with the same basic principle: sample is separated into its structural parts due to the difference in the relative affinities of unlike molecules with the two different phases, mobile and stationary, used in the separation. (laboratoryinfo.com)

In picture 10 are the instruments used on HPLC. The pump sucks and pumps forward the HPLC solvent, which is the mobile phase. The sample is combined with the mobile phase in injector. This liquid is forced through the column, which separates it and the separated compounds are perceived with the detector.



PICTURE 10. Instrumentation of HPLC. (laboratoryinfo.com)

Size Exclusion Chromatography (SEC) is a method that separate analytes purely based on their molecular weight. The molecules will be separated based on their exclusion from pores of the packing material of the column. First will larger analytes elute, in the meantime more interaction will happen between smaller molecules and the stationary phase and they will elute later. (phenomenex.com)

4.2 Kjeldahl

Organic materials such as proteins consists five major elements and nitrogen is one of them. This procedure is based on using strong sulfuric acid to oxidate the organic compound. When the organic material is oxidized the containing carbon is converted to carbon dioxide and the hydrogen is correspondingly converted to water. The amine groups, which exists in the peptide bonds of the polypeptide chains, contain nitrogen which is converted to ammonium ion. This ammonium ion dissolves in the oxidizing solution and it can be converted to ammonia gas afterwards. When calculating the protein content in many different materials varying from food for humans and animals, fertilizer, fossil fuels and waste water the worldwide standard has become the Kjeldahl method of nitrogen analysis. (cuny.edu)

There are three different steps on the Kjeldahl method, which must be carried out in correct sequence:
1.strong sulfuric acid and catalyst is added to the sample. The catalyst helps to convert the amine nitrogen to ammonium ions.

2.the ammonium ions are converted to ammonium gas. The gas will be heated and then distilled. The ammonia gas is redirected into a trapping solution where it dissolves and transforms an ammonium ion all over again.
3.in the end the amount of ammonia that has been captured is determined by titration with standard solution and a calculation is made. (cuny.edu)

Because the crude proteins are being calculated first they must be multiplied with correction factor, to get the right amount of proteins. In the case of plant proteins, the factor is 6,25. This is based on assumption that proteins in food products contain nitrogen 16 %. (ISO 5983-2, 2009, 2; edu.fi)

To calculate the crude proteins, first must be calculated the amount of nitrogen with formula

$$m = M \cdot c \cdot V \quad (1)$$

where
m = amount of nitrogen (g)
M = atomic mass of nitrogen (14 g / mol)
c = concentration of HCl (0,01 mol / l)
V = standard acid – blank acid (ml)

Then this amount of nitrogen is divided by the amount (l) of hydrochloric acid used and this is multiplied with standard value correction factor. (edu.fi)

In the end protein solubility was calculated with formula used in laboratory:

$$Sol_{pH} = \left(\frac{c_s \cdot V_s}{c_i \cdot V_i}\right) \cdot 100\ \% \quad (2)$$

where
c_s = protein concentration in supernatant (g / l)
V_s = final volume of solution after adjustment of pH (ml)
c_i = initial protein concentration (g / l)
V_i = initial volume of solution (ml)

4.3 Phytic acid quantification

The original phytic acid quantification method was developed 1982 but it was modified 1998 and published in 1999 in Food Research International. It is based on the complexometric titration of residual iron (III) after phytic acid precipitation. The ground samples (0,5-5,0g) were extracted for 3 hours at room temperature with 40,0 ml of extraction solution (10 g/100 g Na_2SO_4 in 0,4 mol/l HCl) under magnetic agitation. Then the suspension went to centrifuge at 5000 rpm for 30 minutes and after that filtration was done to the supernatant. The supernatant (10 ml), which contains 3,3-9,0 mg of phytic acid, was pipetted into a 100 ml tube of centrifuge altogether with 10,0 ml of 0,02 mol/l $FeCl_3$ both 10,0 ml of 0,4 mol/l HCl and 10,0 ml of 20 g/100 g sulphosalicylic acid, this was gentle shaken and then sealed with a cork. (García-Estepa, Guerra-Hernándes & García-Villanova, 1999)

A bath for 15 minutes in boiling water was given and then the tube was cooled. Then the sample went to centrifuge at 5000 rpm for 10 minutes, decantation and filtering and the residue of the sample was washed several times with traces of distilled water. Both washed fractions and the supernatant were diluted to 100,0 ml. One sample of 20,0 ml was adjusted to pH 2,5 ± 0,5 with glycine added. The sample solution was heated to 70-80˚C and, when it was still warm, titrated with EDTA solution (50 mmol/l). The phytic acid content was calculated with 4:6 Fe/P atomic ratio. (García-Estepa et al., 1999)

The calculations were made with formulas used in the laboratory:

$$n\,(Fe^{3+})_{free} = V_{eq} \cdot c_{EDTA} \cdot \frac{V_{sol}}{V_s} \qquad (3)$$

where

V_{eq} = equivalent volume (l)

c_{EDTA} = concentration of EDTA (mmol / l)

V_{sol} = volume of initial solution (l)

V_s = volume of taken sample from initial volume (l)

$$n(Fe^{3+})_{precipitate} = n(Fe^{3+})_{blank} - n(Fe^{3+})_{free\ in\ the\ sample} \qquad (4)$$

$$n(Phytic\ acid)_{precipitate} = \frac{n(Fe^{3+})_{precipitate}}{4} \quad (5)$$

$$n(Phytic\ acid)_{extract} = 8 \cdot n(Phytic\ acid)_{precipitate} \quad (6)$$

$$m(Phytic\ acid)_{extract} = n(Phytic\ acid) \cdot M(Phytic\ acid) \quad (7)$$

5 PROCESS IN THE LABORATORY

Process industry can be described with three boxes, shown in picture 11. The first box represents the raw material, in this case it would be rapeseed meal. The second box indicates the production facilities; factory, mill, laboratory etc. In this case the second box is the research laboratory, where solid/liquid extraction and then purification of the proteins takes place. The final third box is the end product from the production facilities. And in this case, it would be the protein isolate.

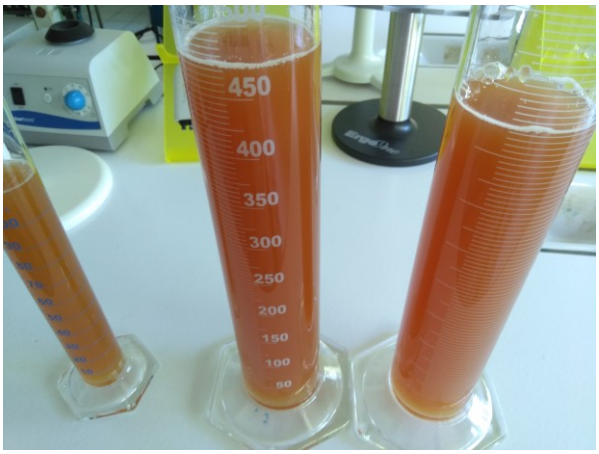
PICTURE 11. Process industry described in three boxes.

5.1 Extraction of proteins – solid/liquid extraction

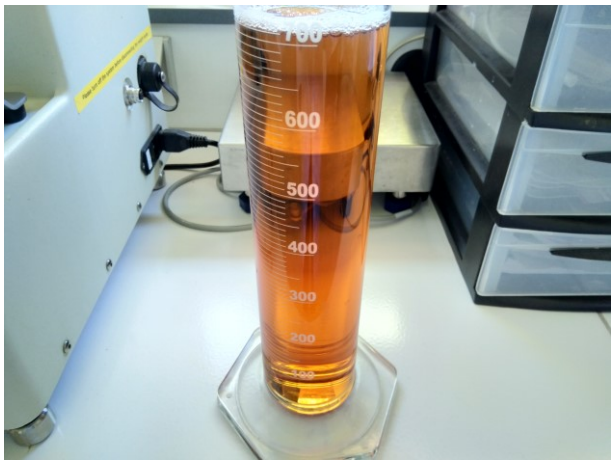
All starts in the laboratory with solid/liquid extraction of rapeseed meal proteins. Pressed rapeseed meal is shown in picture 12. An aqueous mixture is made with it and water and the pH is adjusted with HCl. The low pH in this case will help the wanted protein fractions (albumins) to extract to the water. The solid-to-liquid ratio was 1:9. Usually the rapeseed meal used in the laboratory contains 30-40 % proteins.

PICTURE 12. Rapeseed meal cakes.

After thoroughly mixed in the reactor, the aqueous solution will be given a centrifugation and is filtered after with sieve and with filtering paper. Proteins and other small particles will go through the filters with water and the seed coats and fibres are removed. The aqueous mixture will have a microfiltration, with plate and frame membrane with 0,22 µm pore sizes, just to remove the oil from it. A tangential flow filtration was used. In picture 13 is the mixture before microfiltration and in picture 14 is the filtered clear mixture.



PICTURE 13. Aqueous solution before microfiltration.



PICTURE 14. Aqueous solution after microfiltration.

This phase takes a lot of time and a lot of different phases. The mixture will be given a heat treatment and after it will get a centrifugation again. The precipitate in the centrifuge bottles are the fibres from the rapeseed. After all these phases the mixture is filtered with filtering papers and finally with ultrafiltration to purify the proteins from the rest of the aqueous mixture.

5.2 Purification of proteins – ultra- and diafiltration

With ultrafiltration it is possible separate proteins from aqueous mixture. Ultrafiltration separates bigger molecules, like proteins, retaining them to the retentate, from smaller molecules which are normally impurities allowing them to go through the membrane to the permeate, the waste. Sometimes these impurities, phytic acid and polyphenols, are interacted with proteins which enables them to escape to retentate with proteins. Ultrafiltration was done with hollow-fibre membrane, with 3 kDa pore sizes.

Because this is a research laboratory different membrane options, with different pore sizes, are tested among different conditions of the aqueous mixture, meaning for example different salt concentration. The aim is to find that kind of a conditions that maximize the retention of proteins in the retentate and at the same time maximize the impurities in the permeate.

A retention rate of a membrane is the measure, which tells how well the purifying process success with different membranes. The samples are taken in the beginning of this process and in the end. These samples can be analysed with HPLC and with the given results a ratio can be calculated, which tells the retention rate.

Retention rate with proteins should be as close to one as possible. Some results were 0,955; 0,98; and 0,96. The membrane had different pore sizes every time and the salt concentration were different. With the lowest retention rate 0,955 there were no salt at all. In this field, this kind of a result is too low.

Diafiltration buffer can be water or water + NaCl. NaCl helps to keep apart the proteins from phenolic compounds and from phytic acid. Diafiltration is a special model of ultrafiltration, which washes the impurities away with buffer. All progress can be followed with the HPLC.

5.3 Analyzing the protein isolate with HPLC and Kjeldahl method

Although mentioned in the headline that protein isolate analysis, both methods are being used through whole process. And they both help monitoring how well the extraction and purification is succeeded.

5.3.1 HPLC

Proteins are being analysed with the HPLC, but whatever is done in the laboratory, samples are always taken before and after. These samples are analysed and from the results is easy to see how well the extraction, separation and purification is being succeed. A special analysed method called SEC, size-exclusion chromatography, was used for this purpose.

5.3.2 Kjeldahl

With this method highly dangerous chemicals were used, so safety issues were specially under consideration. Every step was followed carefully and quietly. First step was the mineralization, where all organic material was distracted to inorganic material with catalyser, 97 % sulfuric acid and with high temperature. There were all together 12 samples which two of them were blanks. All sample solution had different pH. When all the organic material was distracted all 12 Kjeldahl method flasks were cooled with air. There was only ammonium sulphate left in the flasks.

When the flasks were cooled to the room temperature the next two steps started. Distillation and titration were done automatically with this KjelFlex K-360 and 877 Titrino Plus device shown in picture 15. Water and 32 % sodium hydroxide were added, and the distillation took place. The distillation released the ammonium from ammonium sulphate.

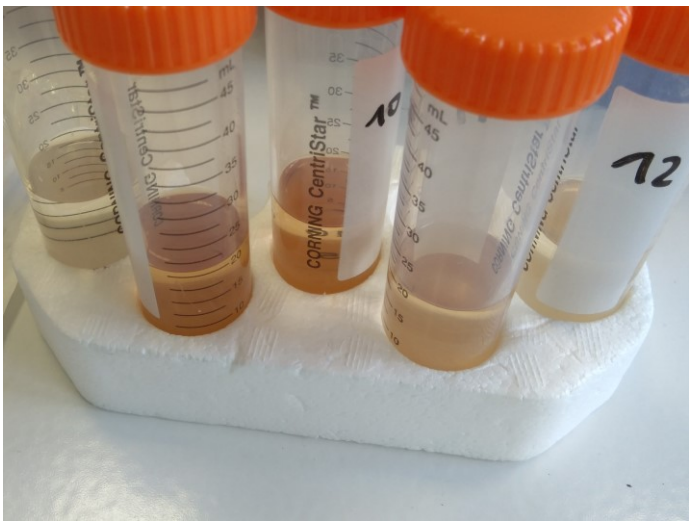
PICTURE 15. The distillation and titration unit of Kjeldahl method.

Boric acid was added to the ammonium solution and this solution were titrated with hydrochloric acid. The amount of nitrogen is calculated based on the consumption of the acid. The apparatus gave the amount automatically. This result will be calculated with correction factor to get the right amount of proteins in a specific species.

5.3.3 Phytic acid quantification

In a diafiltration process a phytic acid is extracted and afterwards freeze-dried. Then the phytic acid is being crushed and this sample powder is quantified. A method used in laboratory has five steps: extraction, reaction, recovery of Fe^{3+} ions, dosage of free ions Fe^{3+} and calculations.

Five samples and one blank were done. These samples were collected from different points of the process. Solid sample was put to beaker and 20 ml of solution (HCl 0,4M + 10 % Na_2SO_4 (w/v)) was added. This will extract the phytic acid from the powder. This mixture was stirred for two hours. Then all the beakers were poured to falcons and put to the centrifuge for 30 minutes. After that the supernatant was filtered. The falcons are shown in picture 16.



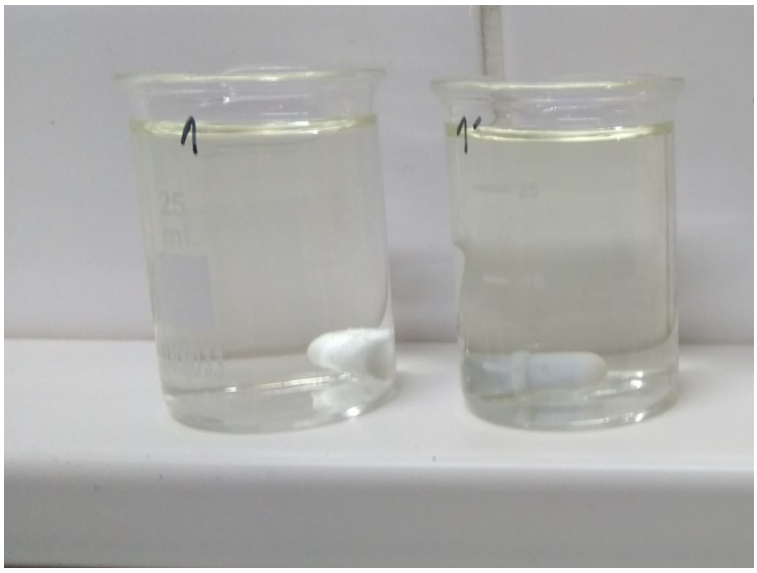
PICTURE 16. Falcons with phytic acid solution.

The supernatant and three reagents were pipetted to the next falcons. Blank was made of reagents only. The solution changed its colour to burgundy immediately. All falcons were given a 100˚C water bath for 30 minutes. During this time a precipitate was formed, which contained sulfosalicylic acid, Fe^{3+} ions and phytic acid. After the falcon tubes were cooled, the tubes with suspension were put to centrifuge again for 30 minutes.

In the next step the supernatant was filtered to volumetric flask. Distilled water is added to the falcon tubes which contains the precipitates. All the phytic acid from the precipitates is wanted. Falcon tubes went to centrifuge for 10 minutes and this cleansing was repeated three times. In picture 17 is volumetric flasks with supernatant and those flasks were filled with water.

PICTURE 17. Burgundy coloured phytic acid after reaction.

Volumetric flasks in picture 17 were stirred and 20 ml of solution from each flask was placed to beaker. The pH of the solution was adjusted to 2,5 ± 0,5 using glycine powder. The solution was heated in 70-80˚C water and when it was still warm it was titrated with EDTA solution. The equivalent volume was reached when the solution changed its colour to yellow-green. In picture 18 are two beakers with this colour solution.



PICTURE 18. Equivalent point of phytic acid quantification.

After all these chemical reactions, the final part was to calculate the phytic acid content in mg / g of protein.

6 RESULTS

The data collected and presented here in the beginning is from the diafiltration process. In the beginning samples are taken from the initial solution. Results in the table 1 are averages from three experiments. After one diafiltration samples are taken again and this is repeated five times, because the solution is diluted five times. Samples are analysed with HPLC and the results are in table 1. From these results a retention curves are drawn to be seen in figure 3. With these experiments the NaCl concentration was 0,35 M.

TABLE 1. Result of diafiltration (from HPLC).

	Protein g / l	Phytic acid mg / g
initial concentration (C_0)	5,06	2,08
DV1 concentration	4,31	1,15
DV2 concentration	4,00	0,64
DV3 concentration	3,77	0,45
DV4 concentration	3,57	0,42
DV5 concentration	3,41	0,31

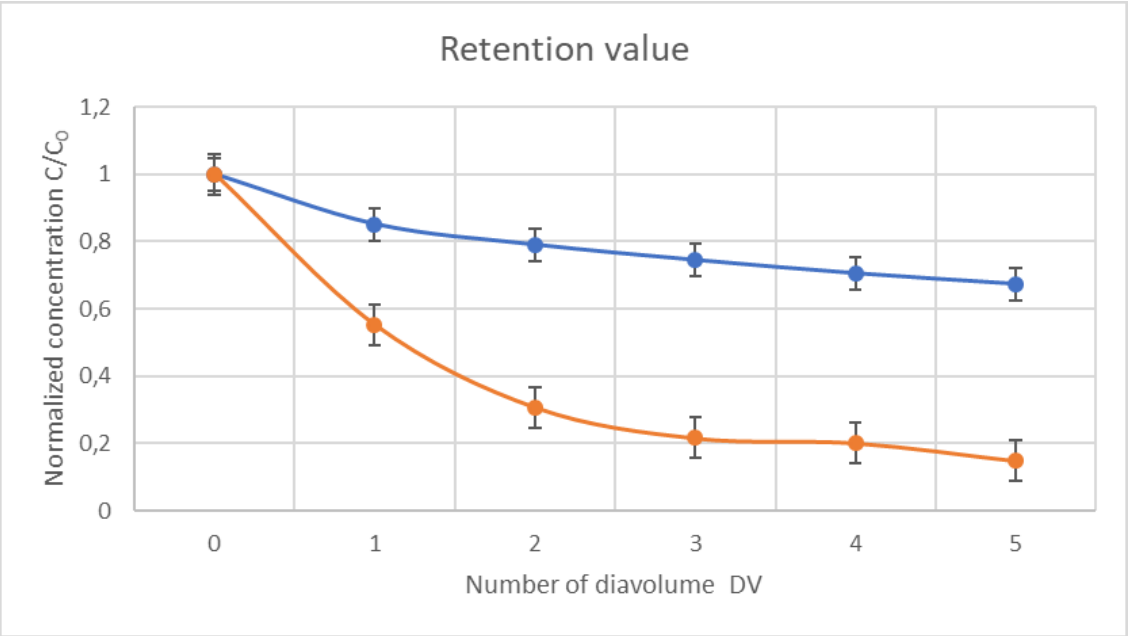


FIGURE 3. Retention value of diavolumes.

Retention value for proteins, blue curve, should be as close to one as possible. And as seen in the figure 3, the amount of phytic acid, orange curve, decreased considerably, which is what is wanted. In this case some of the proteins is lost as well, which is not wanted. A smaller pore sizes of the membrane would probably help with this.

6.1 Isolate characterization – Purity

In figure 4 is the results of purification of proteins with diafiltration. The black curve presents the initial solution and the pink curve is the result from retentate after fifth diafiltration purification. As it is possible to see in the figure 4, proteins can be seen between 8-13 minutes. After 15 minutes the curve shows the amount of phenolic compounds. Based on literature it is common knowledge that proteins can be seen with 214 nm wavelength.

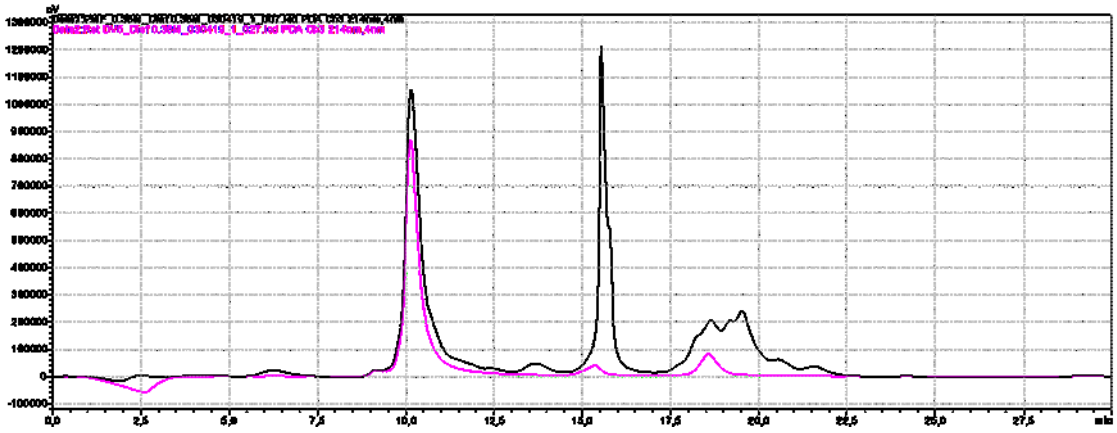


FIGURE 4. Protein purification after diafiltration.

More on phenolic compounds in appendix 5. But as seen above, bigger parts of phenolic compounds have gone to permeate, and the solution has been purified almost completely. Also, some of the proteins has been lost, that is not wanted. The initial solution, black curve, and the retentate of DV5, pink curve, should draw the same curve with proteins. When they don't draw the same curve, some of the proteins pass the membrane to the permeate. This is not desirable, smaller pore size of the membrane would probably help with this.

6.2 Protein solubility

Kjeldahl method was used to find out protein solubility. Calculations were made with formulas 1 and 2. Because in this case the protein source was sunflower, the correction factor was 5,6. The amount of hydrochloric acid used was 500 ml. Blank apparel was tested three times and an average was counted:
V (blank apparel) = (0,914 + 0,906 + 0,936) ml : 3 ≈ 0,91867 ml
V = standard acid – blank acid (ml) = (3,262 – 0,91867) ml = 2,3433 ml

Numerical values were inserted to formula 1

$$m = 14\frac{\mathrm{g}}{\mathrm{mol}} \cdot 0{,}01\frac{\mathrm{mol}}{\mathrm{l}} \cdot 2{,}3433 \cdot 10^{-3}\,\mathrm{l} = 0{,}000\,328\,066\,\mathrm{g}$$

Then this mass was divided with 500 ml amount of HCl and multiplied with correction factor, in this case it was 5,6. This gave the result of protein concentration = 3,674 g/l.

After this the protein solubility was calculated with formula 2 and all the results are in table 2. Also, a curve was drawn and is shown in figure 5.

c_s = 3,674 mg /ml
V_s = 17,8 ml
c_i = 5 mg /ml
V_i = 15 ml

$$Sol_{pH2} = \left(\frac{3{,}674\,\frac{\mathrm{mg}}{\mathrm{ml}} \cdot 17{,}8\ \mathrm{ml}}{5\frac{\mathrm{mg}}{\mathrm{ml}} \cdot 15\ \mathrm{ml}}\right) \cdot 100\ \% \approx 87\ \%$$

TABLE 2. Protein purification as function of the pH.

pH	HCl volume (ml)	Protein concentration (mg/ml)	V_s (ml)	Solubility %
2	3,262	3,674	17,8	87
3	3,624	4,234	15,9	90
4	3,402	3,894	15,39	80
5	2,412	2,342	15,225	48
6	2,092	1,84	15,145	37
7	2,274	2,125	15,065	43
8	2,976	3,226	15,015	65
9	3,538	4,107	15,04	82
10	3,768	4,468	15,2	91
11	3,672	4,317	15,935	92
Blank	3,79			
Blank	3,792			

V_s are from Kjeldahl method, when someone else adjusted the pH to the pH which was reading. They were just told like that.

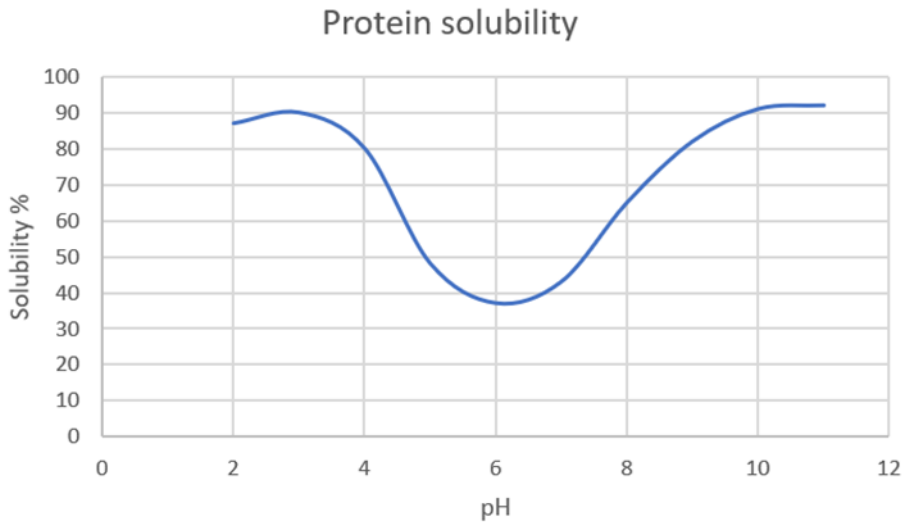


FIGURE 5. Solubility of proteins in different pH values.

This method is used very often, however in this case, the results are from sunflower protein extraction and analyzation process, but the idea is same with rapeseed meal.

Protein solubility is very important, and proteins should be soluble with whatever pH, because the pH for different foods varies greatly. Solubility decreases around and near plant protein's isoelectric point.

6.3 Phytic acid quantification

Quantification of phytic acid was done with formulas 3, 4, 5, 6 and 7. In table 3 are some result from the laboratory, which have been used in calculations. In table 4 are results of the quantification.

TABLE 3. Results from the laboratory to use in calculations.

V_{eq} (ml)	Protein content (%) (from Kjeldahl)	mass of the sample (mg)
10,4	Blank	Blank
9,8	19,8	251,1
6,95	28,1	255,4
7,85	16,3	255,1
8,45	no result	255,9
8,65	21,1	254,9

First was calculated the $n(Fe^{3+})_{free}$ with formula 3

c_{EDTA} = 2 mmol / l

V_{eq} = 10,4 · 10^{-3} l

V_{sol} = 50 ml

V_s = 20 ml

$$Blank\ n(Fe^{3+})_{free} = 10{,}4 \cdot 10^{-3}\,\mathrm{l} \cdot 2\frac{\mathrm{mmol}}{\mathrm{l}} \cdot \frac{50 \cdot 10^{-3}\mathrm{l}}{20 \cdot 10^{-3}\mathrm{l}} = 0{,}052\ \mathrm{mmol}$$

$$n(Fe^{3+})_{free} = 9{,}8 \cdot 10^{-3}\,\mathrm{l} \cdot 2\frac{\mathrm{mmol}}{\mathrm{l}} \cdot \frac{50 \cdot 10^{-3}\mathrm{l}}{20 \cdot 10^{-3}\,\mathrm{l}} = 0{,}049\ \mathrm{mmol}$$

With formula 4 the $n(Fe^{3+})_{precipitate}$ was calculated

$$n(Fe^{3+})_{precipitate} = 0{,}052\ \text{mmol} - 0{,}049\ \text{mmol} = 0{,}003\ \text{mmol}$$

Then with formula 5 the $n(\text{Phytic acid})_{precipitate}$ was calculated

$$n(Phytic\ acid)_{precipitate} = \frac{0{,}003\ \text{mmol}}{4} = 0.00075\ \text{mmol}$$

And with formula 6 the $n(\text{Phytic acid})_{extract}$ was calculated

$$n(Phytic\ acid)_{extract} = 8 \cdot 0{,}00075\ \text{mmol} = 0{,}006\ \text{mmol}$$

With formula 7 the $m(\text{Phytic acid})_{extract}$ was calculated;
M(Phytic acid) = 660 g/mol (IP6 form)

$$m(Phytic\ acid)_{extract} = 0{,}006 \cdot 10^{-3}\ \text{mol} \cdot 660\frac{\text{g}}{\text{mol}} = 3{,}96 \cdot 10^{-3}\ \text{g} = 3{,}96\ \text{mg}$$

TABLE 4. Results of the quantification of phytic acid.

$n(\text{PhyticAcid})_{extract}$ (mmol)	$m(\text{PhyticAcid})_{extract}$ (mg)	$m(\text{Protein})_{extract}$ (g)	**Phytic Acid content per protein (mg / g)**
0,006	3,96	0,0497	**79,68**
0,0345	22,77	0,0718	**317,13**
0,0255	16,83	0,0416	**404,57**
0,0195	12,87	no results	**-**
0,0175	11,55	0,0538	**214,68**

The samples are collected in different points of the process. First sample is from a retentate of DV4. Second sample is from microfiltration permeate. Third sample is from microfiltration permeate with salt. With fourth sample there are no results. And the fifth sample is from DV1 retentate.

Based on the result of phytic acid content per protein and the point of the process it is taken from, it can be interpreted that phytic acid content will decrease through this purification process in the laboratory. And addition of salt will separate phytic acid more.

7 SUSTAINABLE DEVELOPMENT

The world commission on environment and development published a report in 1987 called “Our common future” it is also known as the “Brundtland Report” from the name of the Commission’s chairwomen back then. In that report a sustainable development was defined: “Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs.” (admin.ch)

In picture 19 are all seventeen sustainable development goals of the United Nations mentioned. These are also known as Global Goals to end poverty, to protect the planet, and to underwrite peace and prosperity to all people. These goals were taking effect in 2016 and they are guiding the policy and funding of UNDP (United Nations Development Programme) until 2030. They are currently ratified in 170 countries and territories. (undp.org)

PICTURE 19. United Nations sustainable development goals. (undp.org)

8 CONCLUSION

The world is changing. There are these so called global mega trends that impacts to our business, cultures, economy, personal life, and society defining our future. Some examples of these mega trends are urbanization and mega cities, rise of the middle class, the mass markets and family structures are changing; single person households are the household profile which will grow the fastest. Satellite technology, virtual world, and robotics will also allow online shopping and take away culture to grow. Reducing greenhouse gas emissions and oil dependence and increasing knowledge of environmental awareness, stated then Minister of Interior in Finland, Kai Mykkänen, in fall 2018 in seminar held in Tampere University of Applied Sciences.

And what is common with these Global Goals and with the mega trends? What do all people need to survive and to have energy to keep on going? – Food. And when predicted the world population to grow from 7,7 billion as from now to 8,5 billion by 2030; 9,7 billion by 2050 and exceed 11 billion in 2100. The growing need for food is large-scale. (un.org)

Here the plant proteins step in. Growing plant proteins doesn't need so much energy than producing animal proteins, the environmental impacts are considerably smaller. And with animal protein, the food for animals must be first produced. There are lots of good quality proteins in many seed plants like rapeseed, sunflower or soybean. So why not eat them straight from the source? There has been a bitter off-taste with rapeseed meal proteins, but now after the German researchers figured out the source of this, kaempferol 3-o-(2‴-o-sinapoyl-β-sophoroside), gives new possibilities to find ways to produce proteins which are neutral in flavour.

Some product ranges like mayonnaises and sausages which are already rich in flavour and colour could utilize easily plant proteins and proteins from rapeseed meal. It was already mentioned in some research that proteins from rapeseed meal make stronger foam than egg white, which enables using them in vegan

bakery products. Also, rapeseed proteins' bioactive properties are under research. Good digestibility is one of the most important criteria so decreasing phytic acid from protein isolates is and has been under research for the past 40 years.

All the antinutritional factors, such as glucosinolates, phenolic compounds and phytic acid must be removed. After ultra- and diafiltration most of the phenolic compounds are separated from the proteins. Phenolic compounds are also studied in the laboratory. This kind of action follows the circular economy principle to prevent waste in products and business models.

Researchers are doing tireless work to find new and better methods. In this laboratory sometimes, the retention rates to eliminate phytic acid in protein content were on the peak, hopefully they stay at that level. Whenever the retention rate with proteins is close to one as possible the result is desired. And if at the same time the amount of phytic acid is decreased things are progressing. Membrane pore sizes should be adjusted just right, and some salt should be added to the aqueous mixture, so all the proteins would stay in retentate, and all the other molecules would pass the membrane to permeate. Protein solubility at whatever pH is one of the goals as well. Because the pH with different foods have often large scale. Isoelectric point effects on this though, protein solubility decreases near its isoelectric point, this is typical to all plant proteins.

There isn't profitable manufacturing production yet with rapeseed meal protein isolates. But because of the imminent food shortage, it would be very important to find a way to produce plant protein isolate with good quality in cost efficiency production method.

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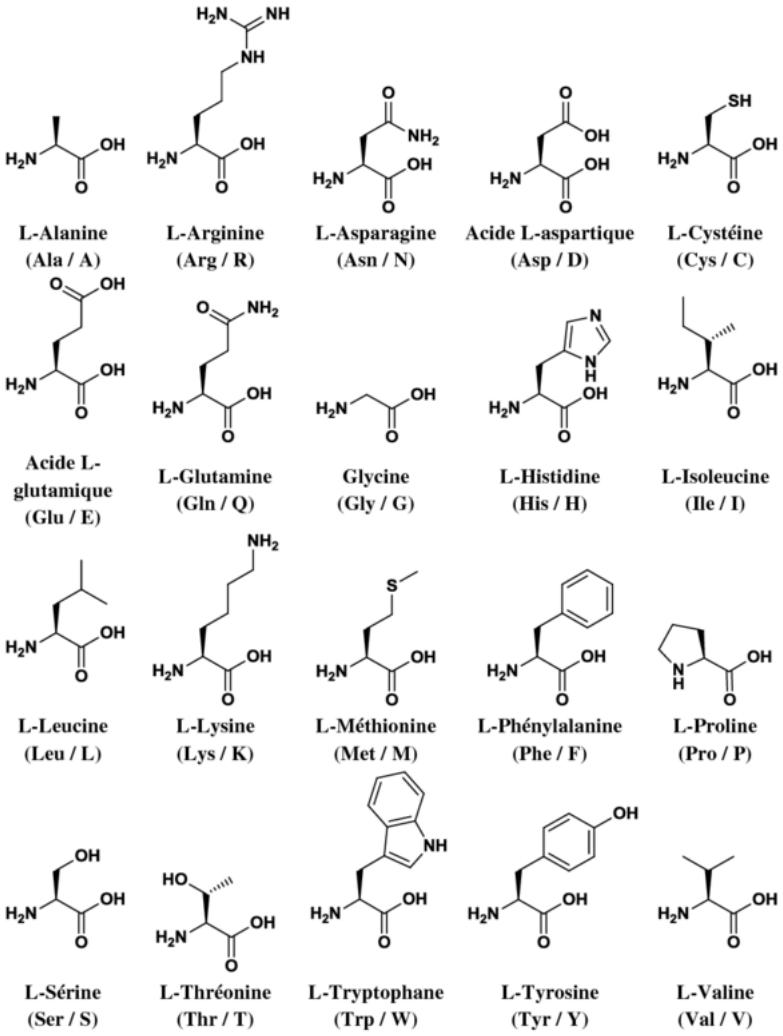
APPENDICES

Appendix 1. Structures of 20 natural amino acids

Structures of 20 natural amino acids (three and one letter codes)



Structures des 20 acides aminés naturels (codes à trois et une lettre). (researchgate.net)

Appendix 2. Molecular weights of amino acids

Amino Acid Abbreviations and Molecular Weights.

Amino Acid	Three-Letter Abbreviation	One-Letter Symbol	Molecular Weight
Alanine	Ala	A	89Da
Arginine	Arg	R	174Da
Asparagine	Asn	N	132Da
Aspartic acid	Asp	D	133Da
Asparagine or aspartic acid	Asx	B	133Da
Cysteine	Cys	C	121Da
Glutamine	Gln	Q	146Da
Glutamic acid	Glu	E	147Da
Glutamine or glutamic acid	Glx	Z	147Da
Glycine	Gly	G	75Da
Histidine	His	H	155Da
Isoleucine	Ile	I	131Da
Leucine	Leu	L	131Da
Lysine	Lys	K	146Da
Methionine	Met	M	149Da
Phenylalanine	Phe	F	165Da
Proline	Pro	P	115Da
Serine	Ser	S	105Da
Threonine	Thr	T	119Da
Tryptophan	Trp	W	204Da
Tyrosine	Tyr	Y	181Da
Valine	Val	V	117Da

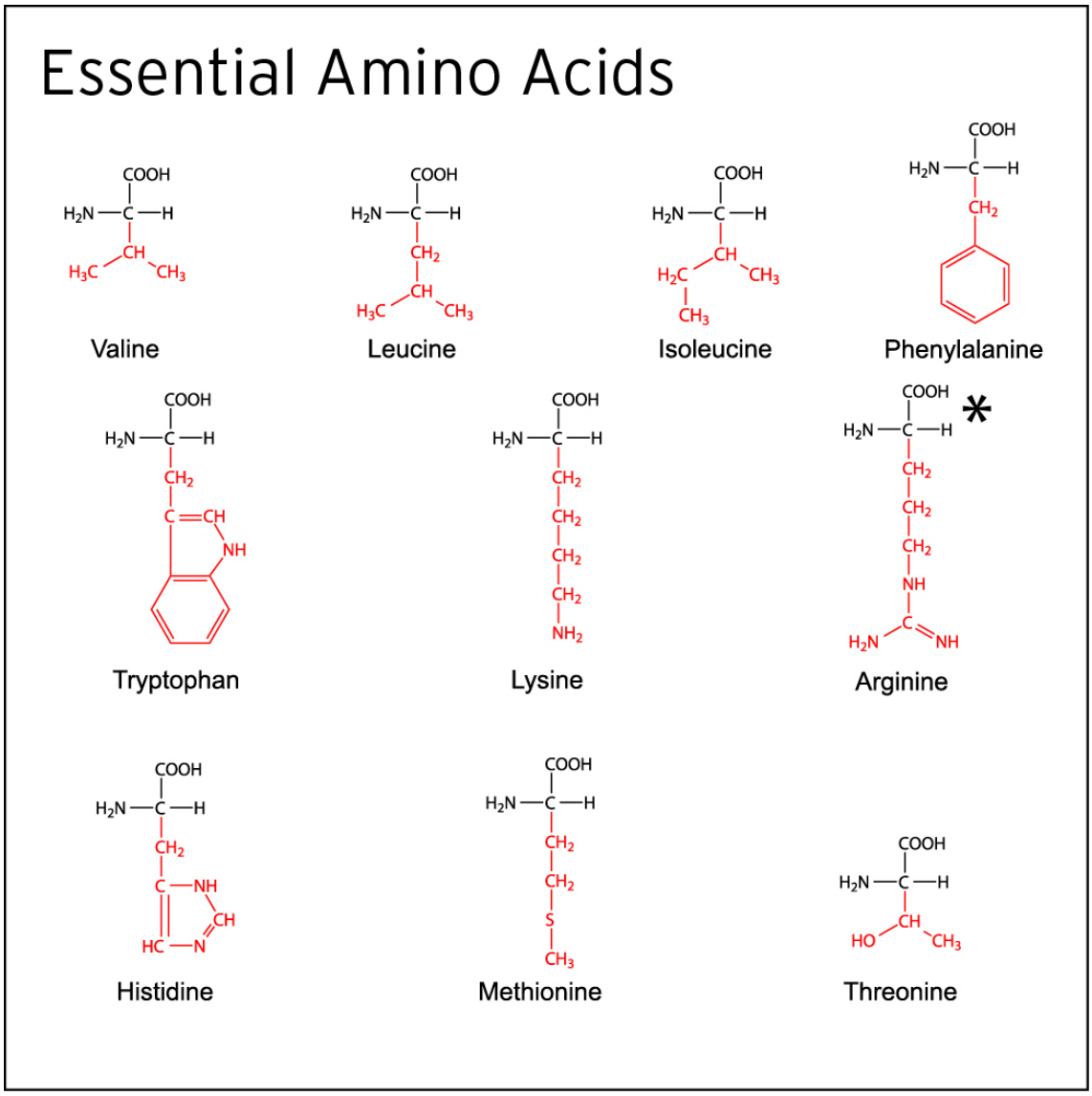
The average molecular weight of an amino acid is 110Da.

Dalton (Da) is an alternate name for the atomic mass unit, and kilodalton (kDa) is 1,000 daltons. Thus a protein with a mass of 64kDa has a molecular weight of 64,000 grams per mole.

(promega.com)

Appendix 3. 9 essential amino acids

(* Sometimes Arginine is considered essential)



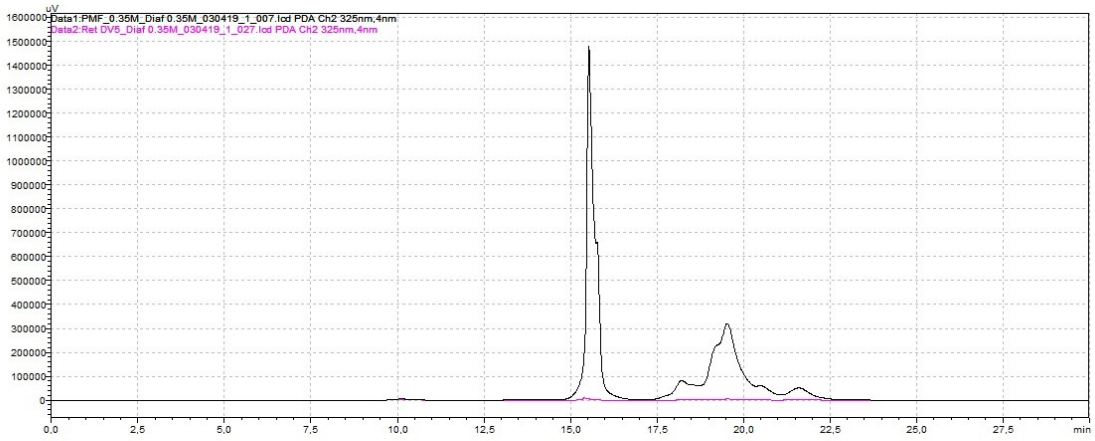
(kulikulifoods.com)

Appendix 4. Variety of functions with the 9 essential amino acids

- Valine prevents the muscles from breaking down.
- Leucine participate regulating blood sugar and recovering of bone tissue and muscle.
- Isoleucine participate healing muscle tissue.
- Phenylalanine is tyrosine's precursor (another amino acid), and the neurotransmitters dopamine (rewarding feeling), epinephrine (adrenaline), and melanin (pigmentation of skin).
- Tryptophan is serotonin's precursor (appetite and mood) and melatonin (regulates blood pressure and sleep).
- Lysine builds muscle, aiding the calcium to absorb, and creating antibodies.
- Histidine is histamine's precursor, which causes inflammation in immune responses.
- Methionine aids new blood vessels to grow.
- Threonine participate in the synthesis of antibodies then helping support strong bones and tooth enamel.

(kulikulifoods.com)

Appendix 5. HPLC analyses for phenolic compounds



The black curve is the initial solution and the pink curve is the retentate after fifth diafiltration. Based on literature it is common knowledge that phenolic compounds can be seen under 325 nm wave length.

The pink curve is almost the same as the x-axis, which indicates that almost all the phenolic compounds has been purified from the proteins after diafiltration. They are collected from the permeate and studied as well in the laboratory.