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Comparison between protein yield in different spring-rape varieties

Jämförelse av proteinutbyte mellan olika vårrapsorter

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Abstract

The market for new protein sources for food has never been as popular as it is today. This is partly a response to the increased climate threat. One major contributing factor to the climate impact is food production and the huge waste that comes from both producer and consumer. Trying to develop new sources of protein is important since carbon dioxide emissions from the protein sources consumed today, often from animals, are very high. Plant protein has considerably lower carbon dioxide emissions and is therefore a good alternative.

Rapeseed is a well-established oil plant that is grown both in Sweden and internationally, especially in Canada. In Sweden, about 108.000 ha are currently grown with rapeseed of both spring and autumn varieties. The crop can be grown on the major parts of southern Sweden and is relatively easy to grow. Rapeseed consists of 30–40% oil and 20–25% protein. The protein in rapeseed has a high-quality amino acid composition compared to other vegetable protein and animal protein as it contains several essential amino acids. Antinutrients such as glucosinolates, phytic acid and erucic acid have long been a problem in rapeseed but thanks to intensive plant breeding, the antinutrients have drastically decreased and are today under approved levels.

Rapeseed contains high-grade protein and is suitable for further processing. Oil extraction is the most common human-consumed product of rapeseed, but the production also results in a by-product, a high-protein content press cake. The press cake is commonly used for animal feed.

In this study, eleven spring rapeseed cultivars were examined, whose protein exchange in the press cake was compared. The study was a part of an extensive research project on rapeseed protein at Lund University. The results showed differences between the protein yield of the varieties and the variety SW Y2909 was found to be the highest yield with 33% crude protein and the lowest yield was Lennon with 13%. A conclusion based on the result is that rapeseed varieties have difference in protein yield.

Keywords: rapeseed, canola, protein, animal protein sources, herbal foods, protein-rich foods, carbon dioxide emissions

Sammanfattning

Marknaden för nya proteinkällor för livsmedel har aldrig varit så efterfrågad som idag. Detta kommer delvis om ett svar på det ökade klimathotet. En stor bidragande faktor till klimatpåverkan är livsmedelsproduktionen samt det stora svinn som blir från såväl producent som konsument. Arbetet för att ta fram nya proteinkällor är viktigt då koldioxidutsläppen från de proteinkällor som konsumeras idag, då ofta animaliska, är mycket höga. Växtbaserade protein har mycket lägre koldioxidutsläpp och är därför ett bra alternativ.

Raps är en väletablerad oljeväxt som odlas både i Sverige och internationellt, framförallt i Kanada. I Sverige odlas idag cirka 108 000 ha raps av både vår- och höstvariant. Grödan kan odlas på större delarna av södra Sverige och är relativt lättodlad. Rapsfrö innehåller 30–40 % olja och ca 20–25 % protein. Proteinet i raps har en högvärdig aminosyrasammansättning jämfört med andra vegetabiliska proteinkällor och animalier samt innehåller flera essentiella aminosyror. Därför lämpar sig raps bra för både human och animalisk konsumtion. Antinutrientier som glukosinolater, fytinsyra samt erukasyra var länge ett problem i raps men tack vare intensiv växtförädling har dessa drastiskt minskat och är idag under godkända nivåer.

Raps är en gröda som odlas i stor utsträckning i världen och innehåller högvärdigt protein och lämpar sig bra för vidare förädling. Oljeutvinning är den vanligaste humankonsumerade biprodukten från raps, men produktionen medför även en restprodukt, en presskaka med högt proteininnehåll som vanligtvis används till djurfoder.

I denna studie undersöktes elva vårrapssorter vars proteinutbyte jämfördes och är en del i ett omfattande forskningsprojekt på rapsprotein vid Lunds Universitet. Resultatet visade att det finns skillnad mellan sorterna och sorten SW Y2909 utmärkte sig som högst avkastande med 33% råprotein och Lennon avkastade lägst, 13%. En slutsats baserad på resultatet är att olika rapssorter har skillnad i proteinutbyte.

Nyckelord: Raps, protein, nya livsmedel, animaliska proteinkällor, växtbaserade livsmedel, proteinrika livsmedel, koldioxidutsläpp

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1 Introduction

Rapeseed is an oil seed plant usually known as the base for extracting rapeseed oil – used in plenty of kitchen worldwide. Globally, rapeseed is the third most cultivated oilseed plant, and Sweden represents for 0.4% of the world production (Nationalencyklopedin 2019b). Rapeseed is grown in all parts of the world, and Canada was the largest producing country in 2012, with 24 % of the global rapeseed production. After Canada, China came with 22 %, France with 8 % and Germany with 7%. In Sweden, approximately 108.000 ha is used for rapeseed cultivation today, and most is for oil production (Jordbruksverket 2018). In 2018, the total harvest of rapeseed was 214.700 tonnes, which is 43% less than 2017 and 35% less than the five-year average. The reduced yield is believed to be caused by the changing climate (Jordbruksverket 2018). The by-product of this pressing process is a press cake that mainly is used as animal feed (Bioenergiportalen 2007). Thanks to intensive plant breeding, there is huge potential for developing protein-rich foods from the press cake for human consumption (Jansen 2018). This is because of the high protein value combined with the low carbon emissions that production contributes to (compared to other protein sources).

1.1 The aim

The aim with this work was to compare eleven spring-rape varieties to determine if the protein yield in the press cake differs between the varieties. The overall purpose is to develop new food products, rich in protein, from the remainders of the rapeseed oil production and this work is part of screening the varieties. Protein was extracted from the press cake and analysed. This study was limited to the protein yield. Factors such as specific amino acid composition and amount of different proteins are not included in this study.

2 Background

2.1 Global health problems and food consumption

The world population is expected to continue growing and with a concomitant increasing need for food. The available cultivable land on earth is limited and is constantly diminishing in line with expansion. The UN General Assembly has agreed for Agenda 2030, consisting of 17 global goals for sustainable development, which are necessary for a sustainable future (United nations 2019). As part of the UN, the Swedish government is working to develop a national food strategy (Regeringen 2019). This strategy aims to contribute sustainable food production, which in turn also will lead to more jobs and economic growth. The goal of the strategy is also to produce food closer to the consumer and an to increase the proportion of organically grown food. In Sweden, the National Food Administration has the Government's mission to contribute to achieving the national environmental goals in the food sector.

Red meat consumption

The consumption of red meat has globally increased during the last decades (Wolk 2017). As part of a healthy and varied diet, red meat is a source of high-quality protein and contains essential nutrients. However, some evidence shows that high consumption of red meat, especially of processed meat, is associated with an increased risk of several major chronic diseases. Furthermore, red meat production is suggested to have a negative impact on the climate. Two-thirds of global methane emissions come from enteric fermentation and one-third from livestock (Moss *et al.* 2000). Fermentation of feed in the rumen is a considerable source of methane emissions. During the fermentation in the rumen, the volatile fatty acids acetate and butyrate are produced which encourage increase the methane production Because of the considerable methane production, animal fats contribute therefore to greater

greenhouse gas emissions than plant-based fats. As a step the direction towards a more sustainable food production, new types of products must be developed (Jansen 2018). Using rapeseed as an energy source via the oil or as a protein source from the press cake is a good alternative. Despite the methane emissions there are benefits of the ruminant animals (Kumm 2011). The Swedish landscape benefits from these grazing animals that keep the land open. An open landscape contributes to increased biodiversity, which is an important asset for the climate. As a step the direction towards a more sustainable food production, new types of products must be developed (Jansen 2018). Using rapeseed as an energy source via the oil or as a protein source from the press cake is a good alternative.

Carbon footprint

Studies show that rapeseed protein has similar carbon footprint as proteins from other legumes, such as peas and soybean (Jansen 2018). The rapeseed protein, like many other plant-based foods, has a lower carbon footprint than poultry and beef. The production of the rapeseed at farm level is where most emissions occur, and the processing of rapeseed oil and the press cake considered to emit less than the production. If protein is extracted from the press cake, further processing is required, which can increase the emissions for the product.

A study by Jansen (2018), shows the difference between the emissions for the production of rapeseed from a Swedish production, Gunnarshög Gård, and an imagined, equivalent production in the Netherlands. The product analysed was cleaned rapeseed with same moisture content of 10.2%, in the both outcomes. The Swedish production at Gunnarshög Gård, where only renewable energy is used, resulted in 0.9 kg CO₂-eq/kg rapeseed, whereas the process in the Netherlands was calculated to give 1.25 kg CO₂-eq/kg rapeseed. This shows that the Dutch production, that used several different fossil-based energy sources, resulted in 39% higher carbon dioxide emissions.

2.2 Antinutrients

Glucosinolates

Originally, rapeseed contained several undesired components such as glucosinolates, phytic acid, and erucic acid (Mejicanos *et al.* 2016). Consumption of food with high levels of glucosinolates can result in thyroid problems. High levels of rapeseed press cake in the feed affect especially poultry and pigs. There is a clear correlation between the amount of glucosinolates in the feed and thyroid

enlargement in the animals. Therefore, not more than 10% of rapeseed in feed for chickens, layer hens and pigs is allowed.

Phytic acid

Just like many other oil seed plants, rapeseed naturally contains phytic acid (Gupta *et al.* 2015). Phytic acid is present as calcium and magnesium salts and the phytates inhibit the uptake of zinc and iron in the intestine. The enzyme phytase does not naturally occur in the intestine of the human body. Very large intake can lead to deficiency diseases.

Erucic acid

Rapeseed oil originally contains 25-45% erucic acid (Mejicanos *et al.* 2016). In the 1960s, animal studies showed that the erucic acid could be stored into the heart (Food Standards Australia New Zealand 2003). Therefore, digesting high levels of erucic acid can lead to diseases such as myocardial lipidosis and heart lesions.

Through plant breeding, the problems with the antinutrients have decreased, and nowadays, the rapeseed is almost totally free from them (Alton *et al.* 1985). Canada has set strict rules for what levels of glucosinolates may be present in the rapeseed. In 1978, the Canadian Oilseed Crusher's Association established the word "canola", the name of the new rapeseed varieties that contain less than 5% of erucic acid in the oil and less than 3 mg/g of glucosinolates in the flour. The requirements became stricter with the years, and since 1996, canola includes all of the oil species of *Brassica* that have reduced levels of erucic acid down to 1% in the oil and less than 20 $\mu\text{mol/g}$ of glucosinolates in the flour.

2.3 Composition of rapeseed

2.3.1 Amino acid composition

The protein in the seeds has a well-balanced amino acid composition, which makes it suitable as an animal feed (Simonsson, 2006). The amino acid composition of rapeseed is similar to the amino acid composition of soy, see table (1). Soybean meal is also a raw material commonly used in animal feed to increase the protein content (Bernesson 2007). The rapeseed contains essential amino acids, including the sulphur containing amino acids methionine and cysteine (Campbell *et al.* 2016). The well-based amino acid composition and the essential amino acid gives the seed a high nutritional value, suitable for both human consumption and animal feed.

Table 1: Amino acid composition in rapeseed, rapeseed press cake and in soybean. The amino acid composition of rapeseed and the rapeseed press cake is the specified per kg dry matter at an oil yield of 60%. For soy, the amino acid composition is specified per kg dry matter.

	Rapeseed (%)	Rapeseed press cake (%)	Soybean (%)
Lysine	1.3	1.7	0.3
Methionine	0.5	0.6	0.6
Threonine	0.1	1.4	1.9
Cystine	0.6	0.8	0.7
Tryptophan	0.3	0.4	0.6

2.3.2 Protein composition

The rapeseed cake consists of 30% of protein. These proteins, in turn, consist of 80%–90% storage protein. Cruciferin (11S globulin) constitute approximately 60% of the total protein content and napin (2S albumin) constitute 25% of the total protein content (Mohamad Ramlan *et al.* 2002). Protein storage vacuoles (PSV) is the primary storage site for these proteins and are found in cotyledon cells.

Cruciferin is a complex, consisting of six monomers and has predominantly β -sheet containing secondary structure (Perera *et al.* 2016). Cruciferin is composed of 465–509 amino acid residues depending on the expressing gene. The tertiary structure is unstable at low pH, and the structure unfolds at pH 3. The solubility behaviour of cruciferin is also affected by the pH changes.

Napin has an α -helical dominant secondary structure and is soluble in a broader pH range than cruciferin and the chemical composition of napin is not as delicate for temperature changes as cruciferin (Perera *et al.* 2016). Napin consists of two polypeptide chains, connected by disulphide bonds (Ericson *et al.* 1986). Comparison of napin and other seed protein shows homology significance between the protein sequence of napin, the castor bean storage protein and a trypsin inhibitor from barley.

Oleosin is an amphipathic oil body protein and is also one of the predominant proteins in rapeseed, constituting 75–80% of the oil body proteins (Mohamad Ramlan *et al.* 2002). Oil bodies are found in plants with high oil content (Hsieh & Huang 2005). Caleosin is also an oil body protein and plays a critical role in the degradation of storage lipids during germination and in lipid trafficking (Shen *et al.* 2016). The two proteins, oleosin and caleosin also act in the cellular structure to prevent coalescence of oil droplets in the rapeseed (Campbell *et al.* 2016).

2.4 Rapeseed processing

2.4.1 Rapeseed oil production

The rapeseed is high in energy and nutrition (Canola council of Canada 2015). The seed consists of 30–40% oil, 20–25% protein and carbohydrates. The oil can either be extracted by heat, so-called hot-pressed rapeseed oil, or without heat, then referred to as cold-pressed rapeseed oil.

Hot-pressed oil

In countries with large-scale rapeseed oil production, solvent extraction is often used (Canola council of Canada 2015). Solvent extraction is done to remove the remainder of the oil from the press cake. Temperatures over 80°C is reached during the process and inactivates the myrosinase enzyme that hydrolyse the glucosinolates. Hydrolysis of glucosinolates results in toxic products such as goitrin, nitriles and cyanates. When pressing, about 50–60% oil is extracted from the seed. By using solvent extraction, the remaining oil can by solvent extraction result in a press cake with less than 1% fat content.

Cold-pressed oil

The maximum achieved temperature during cold-pressing of oil is 35°C–40°C (Knutsson 2007). This method is performed both large scale and small-scale production at farm level. The diameter of the nozzle, the speed of the screw and the water content affects the degree of pressing. Cold-pressing leads to lower oil yield than hot-pressed rapeseed oil (Gunnarshögs Gård 2019). Lower proportion of saturated fatty acids is obtained in the cold-pressed oil than in the hot-pressed oil.

2.4.2 Usage of the rapeseed oil

The main use of rape seed has for long been oil production and feed (Campbell *et al.* 2016). However, there are several other usages for the oil (Nationalencyklopedin 2019b). Rapeseed methyl ester, RME, is a type of biodiesel made of rapeseed oil that has been esterified with an alcohol (Nationalencyklopedin 2019a). The production of RME in Sweden has until 2006 been only small-scale, but as the demand of for renewable fuel has increased, the production has followed.

Rapeseed oil can also be used in the food industry as a lubricant as no health hazardous agents are allowed in the factory that can come into contact with the food that is produced (Fogelfors 2001). Another area of use is as hydraulic oil and for surface treatment of plastic bags. This shows the width of different uses for rapeseed

oil. (Fogelfors 2001) New uses for rapeseed oil are, for example, SCAN's new concept, the "Rapeseed pig" (Scan 2019). The principle of this type of pig production is to add rapeseed oil to the feed, which contribute to an increased proportion of omega 3 fatty acids in the meat.

2.5 Rapeseed press cake

Two-thirds of the amount of the rapeseed used for pressing becomes a press cake. After reducing the levels of glucosinolates and phytic acid (Alton *et al.* 1985), the press cake has many years been used for animal feed. The protein in the rapeseed press cake is high-grade and therefor a good alternative for feed, which is the main area of use (Bernesson 2007). This can be compared to soy flour, which is frequently used in animal feed, soy flour contains more lysine than the press cake. If rapeseed cake is used in the feed, the low lysine content is compensated by high lysine content in cereals. The rapeseed press cake is also used as fertilizer, for combustion and in production of biogas (Bernesson 2007).

2.6 The market for human consumption today and the future

During the 1990s, attempts were made to produce protein-rich foods based on rapeseed (NE.se, 2019). However, this was not a successful project since high levels of erucic acid and glucosinolates was limited. Today, there are still no products based on rapeseed cake as a protein source for human consumption on the market. Not even in Canada, which is the country that produces the most rapeseed, there are protein-based products of rapeseed. Since there are no current products, this topic is particularly interesting and relevant to develop.

An advantage of using rapeseed as a food is that the rapeseed does not contain any common allergens. Soy and eggs are common ingredients in plant-based foods, but since these are typical allergens, they should be avoided in production. A product with no typical allergens, which most can eat, is durable on the food market.

In 2019, Axfoundation and Urban Deli launched a product based on Swedish-grown lupine, field beans (*Vicia faba minor*), grey peas and rapeseed press cake (Jarl 2019). This product only contains these four crops and salt. The base in this product is the sweet lupine and the rapeseed this product is in form of the press cake (Grönsakshallen 2019). The product has just entered the Swedish market and there are no official statistics on the sales and consumer views.

3 Materials and method

3.1 Materials

Eleven different spring rape varieties were used, which were obtained from Lantmännen. The varieties were Majong Cornelis, Kalla, SW X2903, SW X2905, SW Y2907, Mosaik, Lennon, Rasma, Fergus and SW Y2909.

NaOH from Merck KGaA, Darmstadt, Germany

Citric acid from Merck KGaA, Darmstadt, Germany

Aspartic acid from Säntis analytical AG, Schweiz.

Oil press from Oelwerk 100

Knife mill from Retsch, GM 200, GRINDOMAX

N/Protein analyzer Flash EA 1112 Series from Thermo Fisher Scientific

3.2 Oil pressing

In this process, a small-scale press from Oelwerk was used. The aim was, as far as possible, imitate large-scale production of cold pressed rapeseed oil in order to be comparable to industrial conditions. When pressing, the goal is to keep the oil temperature as low as possible. The oil press construction is based on that the seeds are fed into the machine and transported by the press screw to the press head. The seed is pressed through the press head which is heated by a heat collar, and the oil is separated from the remaining press cake. Depending on the size of the nozzle through which the press cake is pressed, different ratios of oil and press cake are obtained.

300 grams of each of the eleven rapeseed varieties was weighed and poured into the press. A nozzle with diameter of 10mm was used and the machine was run at 60

Hz. The press cake was collected, and the weight was noted. The weight and volume of the oil was also recorded where after it was discarded. The press cake was stored in the freezer for further experiments. Three temperature measurements were conducted on the oil and on the press cake during pressing.

3.3 Protein extraction

The press cake from the oil extraction was mixed in a blender repeatedly three times for 20 seconds to avoid heat production. 25 g of the milled rapeseed powder was weighed in beakers and filled up to 250 ml with tap water. The mixture was mixed with a magnet stirrer and pH was adjusted to [REDACTED] by adding 2M NaOH. The mixture was stirred with a magnet stirrer for 4 hours at room temperature. The pH was measured continuously during the first hour and adjusted to pH [REDACTED] when dropped. The solution was transferred to a centrifuge tube and centrifuged at 5,000 g for 20 minutes at room temperature. The supernatant was transferred to a new centrifuge tube and the pH of the supernatant was adjusted to [REDACTED] by adding citric acid. The precipitate was discarded. The supernatant was once again centrifuged at 5,000 g for 20 minutes at room temperature. The two resulted fractions from the supernatant were collected, weighed and kept in a freezer for later use. The whole process from mixing to fractions was done in triplicates.

3.4 Dry matter measurement

The dry matter content was measured in triplicates for all the 33 sediment samples (11 varieties x 3 samples). Approximately 4 g (between 3 and 5 g) sediment sample was weighed into dry matter dishes and the weight noted. The dishes were put in oven for at least 16 hours at 103°C to dry. After drying, the dishes were kept in a desiccator to cool down and were then stored in the desiccator during the weight process. The weight after drying was measured and the dry matter and the water content was calculated.

3.5 Protein analysis

The determination of the nitrogen content was performed using a N/Protein analyzer Flash EA and is based on the Dumas method. The software of is, based on standards, immediately calculating the nitrogen content.

Approximately 40 mg dried sediment sample was weighed into metal foil container and put in separate, numbered modules in a carousel. The carousels were prepared

with the 26 weighed samples, one blank (empty foil container), two containers with aspartic acid, 25 mg and 50 mg respectively (standards) and two samples named unknown 1 and 2, filled with an optional amount of aspartic acid, between 25 and 50 mg. The software was programmed with all sample weight. Aspartic acid was used as standard. The procedure was made in triplicates.

4 Result and analysis

4.1 Oil pressing

Oil was pressed out from rapeseeds with a small-scale press. The average of the weight of the press cake is 189.2 g and most of the cultivars is near the average. There were two outliers, Kalla at 125 g and SW Y2090 at 216.5 g press cake (see table 2). The weight of the oil was in a wider range, from 99.5 to 130.5 g. The average oil weight was 118.9 g. The measured volumes were between 120 ml and 148 ml, and the average volume of the oil was 134.2 ml. Most of the cultivars is near the average.

Table 2: *Weight of the press cake (g) and the oil yield (g) from the eleven rapeseed varieties*

Variety	Weight press cake (g)	Oil yield (g)
Majong	201.5	127
Cornelis	189.5	107.5
Kalla	125	130.5
SW X2903	190	124
SW X2905	193	99.5
SW Y2907	196	130.5
Mosaik	199.5	110
Lennon	194	115.5
Rasma	188.5	121.5
Fergus	188	117.5
SW Y2909	216.5	124.5

The temperature of the oil was between 44°C and 66.5°C and had an average temperature of 51.4°C. The temperature of the press cake was between 69.2°C and 81.4°C and had an average temperature of 73.8°C. The supplied temperature has been constant 80°C to heat the press to about 30°C.

The average temperature of the oil and of the press cake exceeded the limit value of cold-pressed oil. However, the temperature did not reach as high as that of hot-pressed oil. The source to the high temperature is believed to be caused by the model of rapeseed press used. One goal of this study was to emulate large-scale cold pressing of oil. These criteria were not met during the pressing, but the temperature was still low compared to hot pressed oil. If the pressing had been done in large-scale production, other results could possibly be obtained.

The results from the rapeseed pressing should be seen as approximate. The reason for this is that the rapeseed press can never be run empty, the material must be filled through the machine at all times. At all pressures, the machine has been run in a similar manner and gone as close to empty each time to get a comparable result. The total volume of oil and press cake exceeded the supplied volume of rapeseed. The pressing can be made numerous times to ensure the ratio between oil and press cake.

The time for the rapeseed to be pressed affects the oil and rapeseed yield. If a small nozzle is used, it takes a longer time for the rapeseed to be transported through the press, resulting in more oil. The pressing time may vary depending on properties as water content.

4.2 Dry matter analysis

Dry matter of the protein sediment and the rapeseed press cake was measured. The dry matter content in the sediment was between 21%–26%. In the press cake, the dry matter was between 90%–93% (see table 3). The dry matter value for the sediment and the rapeseed press cake has both low standard deviation. The dry matter was measured to conclude if there was correlation between the dry matter and the protein yield from the rapeseed press cake. Since the dry matter for both the sediment and the rapeseed press cake was similar, it was concluded that there was no correlation between dry matter and protein yield in the press cake.

Table 3: *Dry matter in percentage for the sediment and the press cake*

Variety	Dry matter sediment (%)	Dry matter press cake (%)
Majong	25	92
Cornelis	21	91
Kalla	23	91
SW X2903	25	91
SW X2905	25	90
SW Y2907	26	91
Mosaik	22	92
Lennon	22	91
Rasma	23	93
Fergus	23	91
SW Y2909	24	91

4.3 Protein analysis

4.3.1 Protein extraction

There was a clear variation between the sediment sample weight of the eleven rapeseed cultivars. This resulting sediment phase is where the protein exists. The lowest extracted quantity was from the variety Lennon, 9.51 g sediment was extracted (see table 4). There are four varieties, Majong, Mosaic, Fergus and SW Y2909 that was distinguished by giving larger amounts of sediment. The variety that yielded the most was SW Y2909 (18.715 g). The standard deviation indicates on pronounced differences between the tests for some varieties. Lower standard deviation increases the credibility of the results.

Table 4: Protein extraction, sediment (g) and standard deviation

Varieties	Sediment (g)	Standard deviation
Majong	16.81	0.88
Cornelis	13.67	4.09
Kalla	13.81	2.19
SW X2903	11.34	0.59
SW X2905	13.49	0.94
SW Y2907	13.59	0.56
Mosaik	16.07	1.68
Lennon	9.51	1.72
Ramsa	11.27	0.44
Fergus	17.84	1.71
SW Y2909	18.71	1.44

By changing the pH to ■■■, the proteins separate from the scraps which resulted in a cleaner sample. Studies by the research team at Lund University have concluded pH ■■■ as the most suitable pH for this extraction. This value is based on experiments done on the winter-rape varieties from Gunnarshögs Gård. In this experiment, eleven different spring-rape varieties were used which are believed to have similar properties to other rapeseed varieties but cannot be assumed to have the identical properties.

The second pH change was needed to turn the solution acidic and to lower the pH to ■■■ by the addition of citric acid. This step is a critical step in which the proteins in the different varieties react differently. It is possible that each variety has its own pH optimum. This would need to be investigated more. The proteins have different properties and binding capacity which can affect the precipitation properties.

4.3.2 Protein analysis

The amount of protein in both the sediment and the press cake was measured. The variety that yield lowest amount of protein in the sediment was Lennon (10%) and SW Y2907 (15.8%) yield most (see table 5). The variety that yield lowest amount of protein in the press cake was Mosaik (7.5%) and Lennon (10.1%) yield most.

Table 5: Levels protein in the sediment and in the press cake (%)

Variety	Amount of protein in the sediment (%)	Amount of protein in the press cake (%)
Majong	14.8	9.3
Cornelis	10.2	8.0
Kalla	11.7	8.7
SW X2903	14.3	8.2
SW X2905	14.8	8.8
SW Y2907	15.8	8.6
Mosaik	11.7	7.5
Lennon	10.0	10.1
Rasma	13.5	8.2
Fergus	12.9	8.4
SW Y2909	15.6	8.8

There was a distinct difference in protein yield between the eleven varieties. The variety that gave the lowest protein yield was Lennon (13%) (see table 6). The variety that yielded the highest was SW Y2909 (33%). The standard deviation indicates pronounced differences between the tests for some varieties. Lower standard deviation increases the credibility of the results. Although SW X2909 has the largest standard deviation, it gave the highest protein yield.

Table 6: Protein yield in percentage, standard deviation for the protein yield from the rapeseed press cake

Variety	Protein yield (%)	Standard Deviation
Majong	31	0.03
Cornelis	18	0.06
Kalla	19	0.04
SW X2903	20	0.02
SW X2905	26	0.06
SW Y2907	26	0.01
Mosaik	24	0.03
Lennon	13	0.02
Rasma	18	0.05
Fergus	30	0.01
SW Y2909	33	0.09

4.4 Discussion

The aim with this report was to investigate if a difference in protein yield between different varieties of rapeseed can be confirmed. The laboratory work was carried out at the Chemistry Centre, Lund University.

There was a clear difference in protein content and oil content between the rapeseed varieties. In order to confirm the result, more studies should be done to avoid that sources of error lead to misleading results. A source of error can be the pressing method, since in this experiment a small-scale press is used, instead of a large-scale press that are used in industries. Although these presses are equally structured, it can still differ in the result of the proportion between the oil and the press cake. The heat during pressing is believed to vary more in the small-scale press than a large-scale press. This can affect the denaturation of the protein and the fatty acid composition. Another source of error may be the pH adjustments which are a critical step in precipitating the largest quantity of protein. Since the properties of the proteins can differ between the varieties, it is important to investigate this further so that the best possible recovery can be achieved.

The water-holding capacity was discussed when the frozen sediment samples were thawing, various water-retaining abilities were detected. The water-holding capacity may be useful for further studies as it would be of interest to the food industry. The ongoing rapeseed research aims to produce data and examine the possibilities of creating a vegetable meat substitute. This product will probably be stored frozen if it is going to be a commercial food sold in the store. One reason for this is to increase sustainability and reduce food waste, which is a more sustainable product.

The proteins ability to form a gel structure should also be investigated when texture is important in food production.

Before further laboratory work in the area, the following can be improved and seen as sources of error. The impact of the water content to the pressing yield is not included in this study. Factors that can affect rapeseed properties are the cultivation process. This can vary from year to year, depending on the climate conditions and the effort of the farmer.

When cultivating a crop for laboratory investigations, it is important with equal cultivation conditions for all varieties. This is because different factors should not be able to influence and create a difference on the crop. The varieties that have been used for this study are all cultivated in the same place, sown at the same time and treated in the same way regarding fertilizer and chemical control. The cultivation season, 2018, was extremely dry and warm. This affected the harvest as it became much lower than normal.

The rapeseed yield may have an impact if the variety is economically viable or large-scale cultivation and production. The experimental culture has only been done once, so no data is available for comparison the yield.

The results state that the dry matter of neither the rapeseed press cake nor the sediment has any influence on the protein yield. The water content of the seed can affect the weight of the seed. It is the weight and not the volume used in determining the amount of rapeseed used in the pressing. This can be a contributing factor to the various results of the rape pressing.

4.5 Conclusion

The conclusion is provided difference in protein yield between the different rapeseed varieties. This result can be used as a basis for further studies in the area as it demonstrates the difference between the varieties. For both economical and sustainable reasons, a variety of major protein yield is desired.

Another conclusion is that the method worked and that interesting discoveries were made regarding the water-retaining and thawing ability that should be investigated further. These are important properties, as the research hopefully leads to a market-active and climate-smart food product.

References

- Alton, E.B., Swern, D., Formo, M.W., Applewhite, T.H. & Utah State Univ. (Usa). Faculty Association (1985). *Bailey's Industrial oil and fat products. 4th ed.*
- Bernesson, S. (2007). *Användningsområden för biprodukterna vid pressning och omförestring av rapsolja.* (Rapport - miljö, teknik och lantbruk, 2007:4) Available at: <http://www.vaxteko.nu> [2019-05-08]
- Bioenergiportalen (2007). *Användningsområden för biprodukterna vid pressning och omförestring av rapsolja*
- Campbell, L., Rempel, C.B. & Wanasundara, J.P.D. (2016). Canola/Rapeseed Protein: Future Opportunities and Directions—Workshop Proceedings of IRC 2015. vol. 5 (2), p. 17. DOI: <https://doi.org/10.3390/plants5020017>
- Canola council of Canada (2015). *Canola meal feeding guide, 5th edition.* Winnipeg: Canola council of Canada. Available at: https://www.canolacouncil.org/media/516716/2015_canola_meal_feed_industry_guide.pdf [2019-05-06]
- Ericson, M.L., Rodin, J., Lenman, M., GlimeliusQ, K., Josefsson, L.-G. & Rask, L. (1986). Structure of the Rapeseed 1.7 S Storage Protein, Napin, and its Precursor. *The Journal of Biological Chemistry*, vol. 261 (31), pp. 14576–14581. Available at: <http://www.jbc.org/content/261/31/14576.long> [2019-05-13]
- Fogelfors, H. (2001). *Växtproduktion i jordbruket.* Stockholm: Natur och kultur/LT.
- Food Standards Australia New Zealand (2003). *Erucic acid in food: a toxicological review and risk assessment.* (Technical report series, 21). Canberra: Food Standards Australia New Zealand.
- Grönsakshallen (2019). *Baljväxtfärs - infoblad.* Available at: <http://www.gronsakshallen.se/wp-content/uploads/2019/03/Baljväxtfärs-infoblad-Print.pdf> [2019-05-28]
- Gunnarshögs Gård (2019). *Rapsolja.* Available at: <https://www.gunnarshog.se/rapsolja> [2019-05-06]
- Gupta, R.K., Gangoliya, S.S. & Singh, N.K. (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *Journal of Food Science and Technology*, vol. 52 (2), pp. 676–684. DOI: <https://doi.org/10.1007/s13197-013-0978-y>
- Hsieh, K. & Huang, A.H.C. (2005). Lipid-rich tapetosomes in Brassica tapetum are composed of oleosin-coated oil droplets and vesicles, both assembled in and then detached from the endoplasmic reticulum. *The Plant Journal: For Cell and Molecular Biology*, vol. 43 (6), pp. 889–899. DOI: <https://doi.org/10.1111/j.1365-313X.2005.02502.x>
- Jansen, M.H. (2018). *Life Cycle Analysis of the Cold Press of Rapeseed Oil.* (Master thesis). Lund university and Wageningen University & Research.
- Jarl, E. (2019). *Innovativ baljväxtfärs till Hemköp - HEMKÖPSKEDJAN.* Available at: <https://news.cision.com/se/hemkopskedjan/r/innovativ-baljvaxtfars-till-hemkop,c2800219> [2019-05-28]
- Jordbruksverket (2018). *Skörd av spannmål, trindsäd och oljevaxter 2018 Preliminära uppgifter för riket.* (JO – Jordbruk, skogsbruk och fiske, 2018:1) Available at: <http://www.jordbruksverket.se/webdav/files/SJV/Amnesomraden/Statistik,%20fakta/Vegetabilieproduktion/JO19/JO19SM1801/JO19SM1801.pdf> [2019-05-21]
- Knutsson, H. (2007). *Småskalig produktion och användning av rapskaka* Available at:

http://www.svenskraps.se/kunskap/pdf/00735_smaskalig-rapskaka-knutsson-0711.pdf [2019-05-06]

Kumm, K.-I. (2011). *Den svenska kött- och mjölkproduktionens inverkan på biologisk mångfald och klimat*. (Rapport, 2011:21). Jönköping: Jordbruksverket. Available at: <https://www.cbd.int/agriculture/2011-121/SwedishboardAgriculture2-sep11-sw.pdf> [2019-05-22]

Mejicanos, G., Sanjayan, N., Kim, I.H. & Nyachoti, C.M. (2016). Recent advances in canola meal utilization in swine nutrition. *Journal of Animal Science and Technology*, vol. 58 (7), pp. 1–13. DOI: <https://doi.org/10.1186/s40781-016-0085-5>

Mohamad Ramlan, Maruyama, N., Adachi, M., Hontani, N., Saka, S., Kato, N., Ohkawa, Y. & Utsumi, S. (2002). Comparison of Protein Chemical and Physicochemical Properties of Rapeseed Cruciferin with Those of Soybean Glycinin. *Journal of Agricultural and Food Chemistry*, vol. 50 (25), pp. 7380–7385. DOI: <https://doi.org/10.1021/jf0202537>

Moss, A.R., Jouany, J.-P. & Newbold, J. (2000). Methane production by ruminants: its contribution to global warming. *Annales de Zootechnie*, vol. 49 (3), pp. 231–253. DOI: <https://doi.org/10.1051/animres:2000119>

Nationalencyklopedin (2019a). *Biodiesel*. Available at: <https://www.ne.se/uppslagsverk/encyklopedi/l%C3%A5ng/biodiesel> [2019-05-06]

Nationalencyklopedin (2019b). *Raps*. Available at: <https://www.ne.se/uppslagsverk/encyklopedi/l%C3%A5ng/raps> [2019-04-11]

Perera, S.P., McIntosh, T.C. & Wanasundara, J.P.D. (2016). Structural Properties of Cruciferin and Napin of Brassica napus (Canola) Show Distinct Responses to Changes in pH and Temperature. *Plants*, vol. 5 (3). DOI: <https://doi.org/10.3390/plants5030036>

Regeringen (2019). *En livsmedelsstrategi för jobb och hållbar tillväxt i hela landet - Regeringen.se*. Available at: <https://www.regeringen.se/regeringens-politik/en-livsmedelsstrategi-for-jobb-och-hallbar-tillvaxt-i-hela-landet/> [2019-05-22]

Scan (2019). *Svensk Rapsgris - Scan*. Available at: <https://www.scan.se/vara-produkter/artikel-223/> [2019-05-06]

Shen, Y., Liu, M., Wang, L., Li, Z., Taylor, D.C., Li, Z. & Zhang, M. (2016). Identification, duplication, evolution and expression analyses of caleosins in Brassica plants and Arabidopsis subspecies. *Molecular genetics and genomics: MGG*, vol. 291 (2), pp. 971–988. DOI: <https://doi.org/10.1007/s00438-015-1156-x>

United nations (2019). *The Sustainable Development Agenda - United Nations Sustainable Development*. Available at: <https://www.un.org/sustainabledevelopment/development-agenda/> [2019-05-22]

Wolk, A. (2017). Potential health hazards of eating red meat. *Journal of Internal Medicine*, vol. 281 (2), pp. 106–122. DOI: <https://doi.org/10.1111/joim.12543>

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