

RAPSEED – TREMENDOUS POTENTIAL FOR ADDED VALUE GENERATION? COLZA : HAUT POTENTIEL DE VALEUR AJOUTÉE

Rapeseed use in aquaculture

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Received 16 September 2013 – Accepted 23 September 2013

Abstract – The main problem of the aquaculture sector is the provision of suitable and sufficient fish feed, because the most important protein source in aquaculture, the fish meal, is a limited resource. Due to their high nutritional value the rapeseed proteins have great potential as an alternative protein source for the fish nutrition. Therefore, the aim of this work is to develop a manufacturing process of high quality rapeseed protein concentrates, which can replace the limited marine resource. For this purpose, small pilot scale processing procedures were performed to produce a rapeseed protein concentrate (RPC). The meal for protein extraction was prepared by gentle meal processing. Rapeseed protein fractions were prepared by an aqueous extraction procedure. The obtained protein solution is further purified and then dried. The investigated rapeseed protein extraction process provides RPC with high nutritional value and low levels of antinutritional factors. From the nutritional point of view the produced RPC can be compared with the fishmeal. Its amino acid profile reflects the amino acid demands of fish. The obtained RPC was utilized for experimental setups of fish meal replacement in diets for rainbow trout, turbot, common carp and wels catfish. Experimental results from the conducted feeding trials demonstrate an enormous potential of RPC as protein source in aquafeeds. The highest fishmeal replacement level (up to 100%) was observed in the feeding trials with rainbow trout. Therefore, especially in the nutrition of rainbow trout, RPC was identified as an excellent fishmeal alternative.

Keywords: Rapeseed proteins / protein extraction / aquaculture / fishmeal alternative

Résumé – L'utilisation de graines de colza en aquaculture. Le principal problème du secteur de l'aquaculture est la fourniture d'une alimentation appropriée et suffisante aux poissons, puisque la principale source de protéines de ce secteur, à savoir la farine de poisson, s'avère être une ressource limitée. En raison de leur haute valeur nutritionnelle, les protéines de graines de colza ont un important potentiel en tant que source de protéines alternative destinée à la nutrition des poissons. Ainsi, l'objectif de ce travail est de développer un procédé de fabrication de concentrés de protéines de graines de colza de grande qualité, capables de remplacer la ressource marine limitée. À cette fin, un pilote a été réalisé afin de produire un concentré de protéines de graines de colza (RPC, *Rapeseed Protein Concentrate*). Le tourteau destiné à l'extraction de protéines a été préparé par un traitement doux. Les fractions de protéines de graines de colza ont été préparées selon une procédure d'extraction aqueuse. La solution de protéines obtenue a été ensuite purifiée puis séchée. Le processus d'extraction de protéines de graines de colza étudié fournit un concentré à haute valeur nutritionnelle et avec de bas niveaux de facteurs antinutritionnels. Du point de vue nutritionnel, le concentré produit peut être comparé à la farine de poisson. Son profil en acides aminés reflète les besoins en acides aminés des poissons. Le concentré obtenu a été utilisé pour des essais expérimentaux de remplacement de farine de poisson dans l'alimentation de la truite arc-en-ciel, du turbot, de la carpe commune et du poisson-chat. Les résultats expérimentaux des essais d'alimentation conduits démontrent un énorme potentiel de ces RPC comme sources de protéines dans l'alimentation des poissons. Le niveau de remplacement de la farine de poisson le plus élevé (jusqu'à 100 %) a été observé dans les essais d'alimentation menés sur la truite arc-en-ciel. Ainsi, tout particulièrement dans la nutrition de la truite arc-en-ciel, le RPC a été identifié comme une excellente alternative à la farine de poisson.

Mots clés : Protéines de colza / extraction de protéines / aquaculture / alternative à l'alimentation des poissons

1 Introduction

The aquaculture industry is the world's most important provider of fish for human consumption. Now, forty-six

percent of the world's supplies of fish are produced by the aquaculture sector. According to the Food and Agriculture Organization aquaculture is growing by a rate of 9 percent a year since the 1970s. In contrast, the fish meal, which is the protein source traditionally used in aquaculture diets, production

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is declining because of overfishing. The worldwide supply of fish meal (annual production volume 5–7 million tons) will be not able to cover the needs of the expected future expansion of global aquaculture. Therefore aquaculture is looking for alternative suitable protein sources, which could replace this limited resource.

A useful protein source from the by-products of rapeseed oil production, cake or meal, has been suggested as an alternative to fishmeal for aquafeed use. They have relatively high protein content, which is distinguished by a well-balanced amino-acid composition and a high biological value. Rapeseed is one of the world's leading edible oil crops. In recent years rapeseed production has achieved an annual average growth rate of seven percent. Therefore the protein rich rapeseed meal is widely available and less expensive. Unfortunately the rapeseed meal contains also some antinutritional factors (ANF). Prominent ANF in rapeseed are glucosinolates, phenolic compounds (tannins and phenolic acid), phytic acid and indigestible carbohydrates. These antinutritional substrates can affect negative the fish growth performance and health status. The potential of protein rich rapeseed cake or meal as nutrient in diets of different fish species e.g. common carp (*Cyprinus carpio*), Rainbow trout (*Oncorhynchus mykiss*), turbot (*Psetta maxima*), tilapia (*Oreochromis niloticus*) etc. has been investigated by several authors (Dabrowski *et al.*, 1981; Burel *et al.*, 2000a; 2000b; Yigit *et al.*, 2009). They all discuss, that the nutritional quality of rapeseed products as fish meal substitute largely depends on their level of antinutritional compounds. Therefore they have to be removed or minimized before the rapeseed protein products to be used in fish nutrition.

2 Objective

The present study discusses ways to develop suitable extraction procedures for the production of a rapeseed protein concentrate of high quality, which ensure the full replacement of fish meal protein. The production process is evaluated regarding to the protein quality and yield. The extraction of both main storage proteins of rapeseed, cruciferin (12S globulin) and napin (2S albumin), is considered. By using of different processing techniques the content of antinutritive substances were also adjusted. The nutrient composition, amino acid profile and antinutritional ingredients of the obtained protein product are determined. The produced rapeseed protein fraction with high protein content and low levels of ANF is delivered for study of the effectiveness of these oilseed proteins as alternatives to fish meal. The rapeseed protein concentrate is evaluated as protein sources in diets for rainbow trout (*Oncorhynchus mykiss*), turbot (*Psetta maxima*), common carp (*Cyprinus carpio* L.) and wels catfish (*Silurus glanis* L.).

3 Materials and methods

3.1 Preparation of rapeseed meal

Rapeseed (*Brassica napus*) of variety "Lorenz", provided by Norddeutsche Pflanzenzucht (NPZ), was used as raw material. The seeds were heat treated at 80 °C in a paddle dryer

(type D 600, DVA Holland Merten GmbH) for 15 min to inactivate the glucosinolate degrading enzyme myrosinase in the seeds. The rapeseed was then cold pressed in a screw press (type Komet S87G, Monforts). Hereby, a press head temperature of about 45–55 °C was observed. The press cake with residual oil content of appr. 13% was crushed to particle size of 5mm using a grain crusher from the Egon Sommer Maschinenbau GmbH & Co. KG and further de-oiled within a hexane extraction. The solvent extraction was carried out in the pilot plant for solid-liquid extraction with miscella distillation from the Bio-Engineering GmbH. A two-step extraction by percolation was performed. The extraction was carried out in a closed circuit operation for two hours by a solvent circulation of 500–550 kg/h at 60 °C. After the de-oiling, the residual hexane in the meal was gently removed in our newly-developed small pilot-scale fluidized bed desolventizer (in cooperation with Dr. Weigel Anlagenbau GmbH). The hexan-containing meal was desolventized at about 90 °C (fluidization temperature) for 15 min. The product temperature is thereby between 65 and 80 °C. The desolventized meal was then ethanolic extracted to remove the antinutritive substances like glucosinolates, sinapic acid and other. The ethanolic extraction was conducted in the pilot plant for solid-liquid extraction with miscella distillation, too. The meal was treated by a 75% ethanol solution for 35 min at 60 °C by a solvent circulation of 500–550 kg/h in a closed circuit operation. A four-step extraction procedure was used. After the extraction, the ethanol-containing meal was desolventized by ventilation under ambient conditions. Subsequently, for the protein extraction the detoxified rapeseed meal was milled to a powder with a particle size < 200 µm using roller mills Haferquetsche Rapsmaster 15S from Egon Sommer Maschinenbau GmbH & Co. KG.

3.2 Preparation of rapeseed protein concentrate

Milled rapeseed meal was suspended in 0.5 M NaCl solution in a 1:10 ratio (w/w) and stirred at 45 °C for 2 h in a stirring vessel SWBR 160 E (Apparatebau Burg GmbH). The phase separation of the extraction suspension was performed using a decanter-centrifuge MDZ 004 from Lemitec GmbH. The residue of this suspension was separated at 6500 rpm and was then used as starting material for the second protein extraction step. It was suspended in water in a 1:10 ratio (w/w) and stirred at 45 °C for 1 h by adjusting the pH-value of 9. During the entire extraction time the pH value was kept constant. The phase separation was conducted by decantation, too. The final residue of the 2nd extraction was dispose of.

The supernatants, which contain dissolved protein, from the 1st and 2nd extraction step were collected and further treated by ultra-/diafiltration to remove the salt and other low molecular weight compounds, including antinutrients, and also to reduce the water content. For this purpose, a two-stage UF-apparatus UF-2Wi 4040 from UFI-TEC with two spiral wound membranes arranged in series (Spira-Cel[®] Wickelmodul GY-UP020-4040F from Microdyn Nadir GmbH with a molecular weight cut-off at 20 kDa and spacer of 80 mil) was utilized. The ultra-/diafiltration were carried out till the conductivity of the retentate (protein solution) reaches 5–6 mS/cm. Finally the

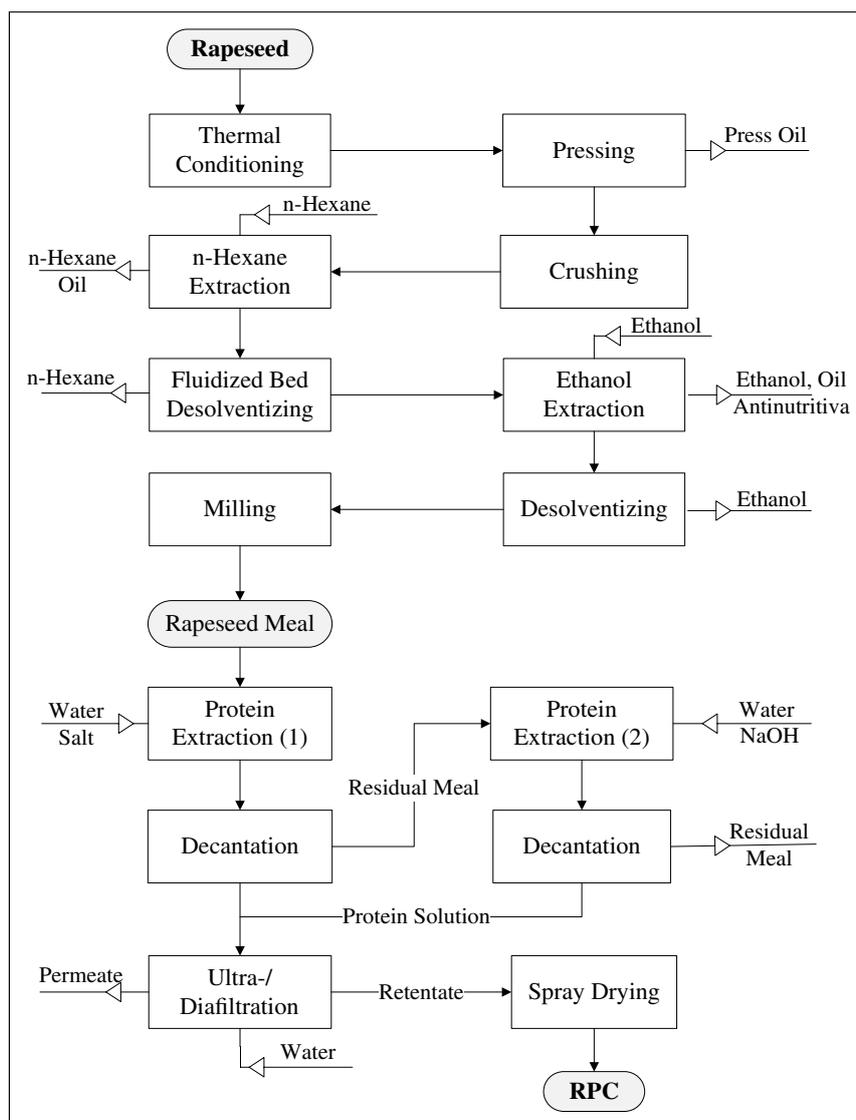


Fig. 1. Process scheme for production of rapeseed protein concentrate (RPC).

purified protein solution was spray dried. The inlet and outlet temperatures range from 120 °C to 130 °C and from 70 °C to 75 °C, respectively.

Figure 1 represents a process flowsheet of the small pilot scale processing procedures used to produce the rapeseed protein concentrate.

3.3 Characterization of rapeseed meal and protein concentrate

3.3.1 Chemical analysis

The dry matter was determined by Sartorius MA 30 moisture analyzer at 105 °C. Crude protein contents were calculated from the nitrogen content (N₂), according to the Kjeldahl method following the official VDLUFA method (VDLUFA-Methodenbuch III, Kap. 4.1.1) with a conversion factor of 6.25. The crude fat content was determined in

accordance with DIN EN ISO 659 (1998–10). The crude fiber content was measured according to the official VDLUFA method (VDLUFA-Methodenbuch III, Kap. 6.1.1). The crude ash content determination is carried out using the official VDLUFA method (VDLUFA-Methodenbuch III, Kap. 8.1). The glucosinolate content and phytic acid content were determined according to the method EG 1864/90 L 170/28 and SAA A 004, respectively. The parameters listed above were analyzed by ÖHMI Analytik GmbH and LUFA Nord-West Institute for Feed Analysis. The analysis of the sinapic acid content was carried out by the company ACT FOODS GmbH according to an accredited in-house method. Amino acid concentrations were analyzed by ÖHMI Analytik GmbH.

3.3.2 SDS-PAGE electrophoresis

The molecular weight of the extracted proteins was determined by SDS-polyacrylamide gel electrophoresis

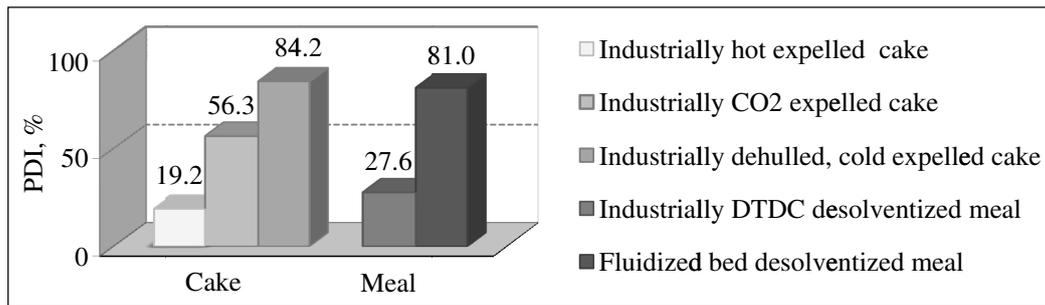


Fig. 2. PDI of rapeseed meal treated by gentle fluidised bed desolventizing compared with industrially produced rapeseed meal and cake.

(SDS-PAGE). The electrophoresis was performed according to the method of Laemmli (Laemmli, 1970). A 14% separating gel with a 1.5 M Tris buffer pH 8.8 and a 5% (w/v) stacking gels with a 0.5 M Tris buffer pH 6.8 were used. A vertical gel electrophoresis unit CTV100YK from VWR for 10 × 10 cm gels was utilized for the electrophoretic analysis. 10 µl of the protein sample was loaded on each lane of gel and run for 2 h at 80 mA at room temperature. After separation, the gels were stained with coomassie blue, and then decolorized with a destaining solution. To determine the molecular weight of the samples, a molecular weight standard from Calibrations Kit Electran (VWR, Prolabo, molecular weight in the range of 12.3 to 78 kDa) was used. The relative quantity of the protein fraction was calculated by means of the GeneTools-software. Based on the relative quantity, the composition of the protein fraction in RPC was determined.

3.3.3 Protein dispersibility index (PDI)

To determine the protein dispersibility index (PDI), the AOCS method Ba 10b-09 was applied in a modified form. After the AOCS method Ba 10b-09, which was developed for soy protein, the rapeseed proteins can not be extracted adequately. The PDI, measured by this method, only covers the globulin fraction of the proteins because PDI determination is carried out in aqueous solutions using distilled water at neutral pH. Therefore, a new method was developed by PPM e.V. with a 3-stage extraction procedure having different extraction conditions with respect to salinity and pH value, which ensures the extraction also of the albumin fraction of the rapeseed proteins. Therefore, using our modified method, significantly higher PDI values can be achieved compared to the standard AOCS method (Pudel, 2011).

4 Results and discussion

4.1 Process design

The most important requirements for the provision of a rapeseed protein product for aquaculture are:

- suitable raw material for protein extraction,
- optimal protein extraction process,
- favorable nutritional properties for fish.

The subsequent economic protein extraction places enormous demands on the starting material, meal or cake. It should have the following properties:

- PDI as high as possible,
- low oil content,
- low contents of ANF,
- low hexane content (<300 ppm),
- particle size (<200 µm).

The most important factor influencing the yield of extraction of the proteins from the starting material is the protein solubility, measured as PDI. Due to minimal thermal load, resulting in low protein denaturation, high PDI values can be achieved. However, during the conversational rapeseed processing high operating temperatures and long operating times were applied. The most critical steps in regard to protein quality are hot pressing and the meal desolventizing. The desolventizing processing step conduces to remove the residual hexane in the deoiled meal (oil content <1%), which is carried out in so called Desolventizer-Toaster-Dryer-Cooler (DTDC) by temperatures of more than 100 °C and retention time of 1–2 h (De Kock, 2007). Therefore, especially during this treatment the proteins are thermally damaged, leading to high degree of denaturation of proteins and thus considerable decrease in the protein solubility, which make it significantly more difficult to extract the proteins from the meal. A reduction of protein digestibility and amino acid availability also occurs during the desolventizing/toasting (Schöne, 2007).

As a result, the extraction of proteins from rapeseed meal produced in the industrial oil mills is indeed possible, but only in low yield with reduced nutritional properties. Therefore, to produce high-quality rapeseed proteins for use in aquaculture, such procedures should be established to ensure a high meal quality. Thus, a new desolventizer based on fluidized bed principle was developed by PPM e.V. By treatment in the new fluidized bed desolventizer the hexane content in the rapeseed meal can be removed up to lower than the target value of 300 ppm without damaging the containing proteins. So that high PDI values of gentle fluidized bed desolventized rapeseed meal can be achieved. The Figure 2 shows the PDI of meal treated in the fluidized bed desolventizer in comparison to other by-products of commercial rapeseed processing.

The innovative gentle meal processing by using a fluidized bed desolventizer improves the meal quality and so increases three times the PDI taking values from 27.6 to 81.0. According to Figure 2 the industrial cake produced by dehulling and

cold expelling have also a high PDI similar to the fluidized bed desolventized meal. But it is still not suitable for subsequent economic protein extraction because of its higher oil content compared to a de-oiled meal. The high oil content can reduce the protein yield due to the formation of emulsions during the aqueous protein extraction (Wäsche, 2002; Natsch, 2006).

Therefore, for own technology development and process design rapeseed meal, which is the worldwide dominant by-product of the commercial rapeseed oil production (Jeroch, 2008; Newkirk, 2009), was chosen as starting material for protein extraction. As described in Section 3.1, to avoid protein damage within the rapeseed processing chain, the seed was de-oiled by cold pressing and hexane extraction following by gentle fluidized bed desolventizing.

The nutritive quality of rapeseed protein products for fish feeding largely depends on their content on antinutritional substances. The glucosinolates and their hydrolysis products can negatively affect the thyroidal functions and the feed acceptance because of their bitter taste, from there the feed intake and correspondingly the fish growth performance (Bell, 1993; Mawson *et al.*, 1994; Burel *et al.*, 2000; Burel *et al.*, 2001). The phenolic acid such as sinapic acid causes also a bitter taste. The phytic acid may have contributed to reduced phosphorus availability in fish (Francis, 2001) These secondary plant ingredients are undesired not only from nutritional point of view, but also by reason of their ability to react with proteins and change their functional properties like solubility, isoelectric point (IP), ratio of hydrophilic/hydrophobic properties, molar weight and other, and also nutritive properties such as digestibility and bioavailability (Kroll *et al.*, 2007). The protein extraction can therefore be affected adversely by these antinutritional factors.

Therefore, the antinutrients especially the glucosinolates in the meal should be removed. It is well known that the glucosinolates and phenolic acids like sinapic acid are soluble in organic solvents such as ethanol, methanol and acetone (Dabrowski *et al.*, 1984; Mawson *et al.*, 1995). In order to reduce the antinutritional ingredients the oil free meal was ethanolic extract in accordance to the method described in Section 3.1. The experimental results demonstrate a reduction of the glucosinolate content by the ethanol treatment at approximately of 85%, so that the glucosinolate content decreases from 20.7 $\mu\text{mol/g}$ to 3.0 $\mu\text{mol/g}$. Therefore, the ethanol treatment is an efficient way to remove glucosinolates from the rapeseed meal. But on the other hand it negatively affects the protein solubility due to protein denaturation (as explained by Fokushima (1969)), so that the PDI is reduced by appr. 40%, this in turn means a decrease of protein yield.

The protein yield depends also on the actual protein extraction. A review of available literature covering the investigated extraction principles and conditions of proteins from rapeseed is conducted by Tan *et al.* (2011) and Xu *et al.* (2013). In fact, the extraction of rapeseed proteins is more difficult compared to the soybean proteins because the two major storage proteins in the rapeseed (cruciferin and napin) are distributed in almost equal parts (cruciferin napin ratio 1.1–1.3 for 00-rapeseed variety (Malabat *et al.*, 2003)) and have different solubility properties. Therefore, from an economic point of view, both protein fractions have to be extracted, which

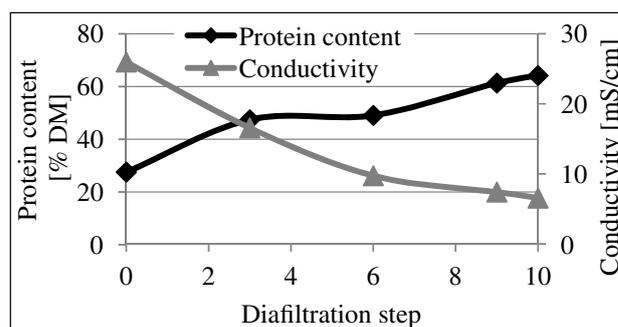


Fig. 3. Protein content and conductivity of the retentate during the diafiltration.

necessitates the adjustment of different extraction parameters in a multi-step process. Hence, a two-step protein extraction process was taken into account for the process development. Different extraction conditions are met (see Sect. 3.2). Extraction by sodium chloride solution (1st extraction) as well as alkali solution (2nd extraction) was carried out to ensure the obtaining of both protein fractions and at the same time to increase the protein yield. In fact, the protein yield could be increased by a downstream second protein extraction by about 70%.

The collected protein solution from 1st and 2nd extraction was subsequently ultra-/diafiltrated to purify and concentrate the dissolved proteins. The next graph illustrates the trend of protein content and conductivity during the diafiltration (Fig. 3). While the salts and other low molecular weight compounds are removed from the protein solution as a permeate, the retained proteins in the so-called retentate are enriched. By multiple step of diafiltration a high purification degree can be achieved. During the diafiltration process the protein content of the permeate increases by more than 100%. Hereby, as a parameter for reached protein purification degree the conductivity of the retentate was measured because it is able to make a statement on the amount of salts still contained in the retentate. For instance, the initial protein solution has conductivity of 26.0 mS/cm that corresponds to a protein content of 27.6% on a dry matter basis. By reducing its value of about 6.6 mS/cm or lower due to removing the salts a protein concentrate with protein content of 64.2% or higher can be achieved. The purified protein solution was then spray dried to produce the final rapeseed protein product.

4.2 Product characteristics

4.2.1 Protein profile

The molecular weight profile of the obtained rapeseed protein concentrate and its composition of protein fractions are shown in the Figure 4. The two major storage proteins in rapeseed are the low molecular weight protein napin (2S albumin) with a molecular weight of 12–17 kDa and the high molecular weight protein cruciferin (12S globulin) with a molecular weight of about 300 kDa. Napin is composed of two polypeptide chains with molecular weights of 9 and 4 kDa, linked by disulfide

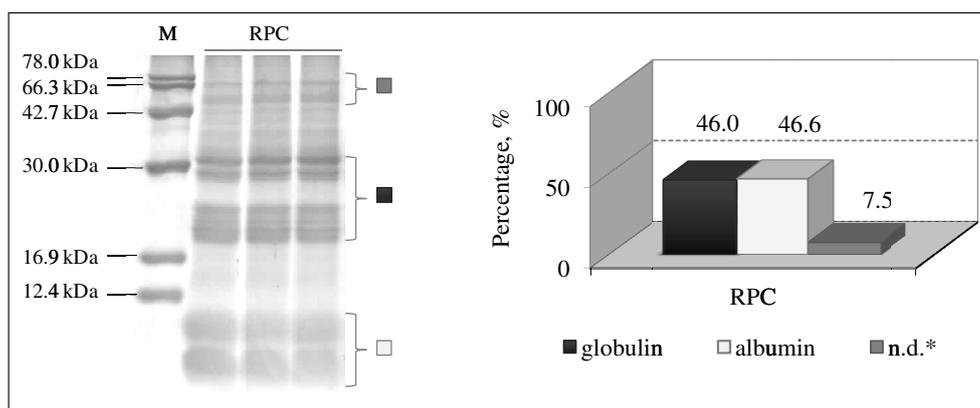


Fig. 4. SDS-PAGE of RPC (left) and composition of protein fractions of RPC (right). Molecular size marker is indicated on lane M of the SDS-PAGE. * Not defined.

bonds. Cruciferin is consisted of 6 subunits and each subunit contains smaller units (polypeptide chains) with molecular weights in the range of 18.5 to 31.2 kDa, linked by disulfide bonds (Schwenke *et al.*, 1973; Schwenke *et al.*, 1983; Mieth *et al.*, 1983; Schwenke, 1990). Based on the SDS-PAGE assay the both storage proteins were observed in the produced protein product concerning the detected bands with molecular weights of 31–29, 24–21 kDa for cruciferin polypeptide chains and 9–4 kDa for napin polypeptide chains. The electrophoretic analysis revealed that the RPC contained the both protein fractions by the same ratio.

4.2.2 Nutritive value

To characterize the nutritive value of the RPC obtained by the developed production method, its proximate composition and amino acid profiles as well as the concentration of antinutritional factors were determined. These results are given in the Table 1.

By extraction of proteins from the meal the nutrient content of the final product was significantly improved. The protein extraction with a saline and alkali solution dialyzing with an UF-apparatus resulted in a protein content of less than 70%. Therefore, the target protein content (this of the fish meal) was reached. The contents of fibre and other carbohydrates (calculated as nitrogen-free extract NfE) were preferably decreased compared to the rapeseed meal. However, the NfE content is still high by contrast with the fish meal. A much higher NfE content such as this of the rapeseed meal can negatively influence protein digestibility in carnivorous fish (Burel *et al.*, 2000a; Francis, 2001). The calculated gross energy of RPC was similar to the fish meal.

To make a better statement about the nutritional quality of the produced protein product, its amino acid composition was investigated. There is little difference in amino acid profile between starting rapeseed meal and RPC (Tab. 1). It can be explained with dependence of the amino acid content on the extraction method. The change in the amino acid profile is connected with the fact that the albumins and globulins were not extracted equally from the meal by the selected extraction conditions because of their different solubility behavior in aqueous

Table 1. Proximate and amino acid composition of rapeseed meal (RM), rapeseed protein concentrate (RPC) and fish meal (FM) and concentration of antinutritional factors detected in RPC.

Ingredients	RM	RPC	FM
<i>Proximate composition (% of dry matter)</i>			
Crude Protein	41.6	71.2	69.0
Crude fat	1.2	0.6	7.0
Crude ash	10.5	16.1	20.7
Crude fibre	11.5	0.5	0.5
Nitrogen-free Extract (NfE)^a	35.2	11.6	2.8
Gross energy^b (MJ/kg)	18.6	19.4	19.9
<i>Essential amino acids (g/100 g crude protein)</i>			
Arginine	6.30	6.78	5.84
Histidine	2.75	3.86	2.00
Isoleucine	4.16	3.79	3.62
Leucine	7.40	7.57	6.45
Lysine	6.30	7.87	6.55
Methionine	2.04	2.36	2.36
(+ Cysteine)	4.31	6.52	3.17
Phenylalanine	4.16	3.60	3.52
Threonine	4.72	4.17	3.90
Valine	5.40	5.15	4.45
<i>Antinutritional factors</i>			
Glucosinolates ($\mu\text{mol/g}$)	3.03	1.32	–
Phytic acid (g/100 g)	3.34	1.77	–
Σ SAE^c (g/kg)	not determined	0.13	–
thereof SA (g/kg)		0.04	
thereof SIN (g/kg)		<0.01	
thereof SG (g/kg)		<0.01	

^a Nitrogen free extract = 100 – (%crude protein + %crude fat + %ash + %fibre).

^b Calculated by: crude protein = 23.9 MJ/kg; crude fat = 39.8 MJ/kg; NfE, fibre: 17.6 MJ/kg.

Tryptophane was not analyzed.

^c Σ SAE = sum of sinapic acid and sinapic acid ester.

SA = sinapic acid; SIN = sinapin; SG = sinapoylglucose.

media. Therefore, the albumin globulin ratio of the RPC corresponds not anymore to the initial ratio in the meal. Due to the different amino acid sequence of the both proteins the produced RPC may have a slightly different amino acid composition from the starting meal. For example, napin is rich in lysine (the most limiting amino acid in all oilseed proteins) as well as sulfur containing essential amino acids like methionine and cysteine (Ericson *et al.*, 1986; Schwenke, 1990; Wanasundara *et al.*, 2010). Exactly the content of these amino acids has increased in the RPC dramatically.

According to the Table 1 the RPC is an excellent source of all essential amino acids. They have amino acid levels equal to (by methionine) or greater than fishmeal. The amino acid profile of RPC reflects well the fish amino acid needs which could imply higher substitution levels.

The quality of the rapeseed protein product is determined to a large extent also by the content of antinutritive substances. The reduction of the antinutritive compounds provides a successful replacement of fishmeal. As shown in the Table 1, the produced RPC has reduced content of ANF due to the ethanolic treatment of the rapeseed meal. The lowering of the glucosinolate, phytic acid and sinapic acid level is also associated with the ultra-/diafiltration step as the toxic compounds have significantly lower molecular weights than rapeseed proteins (Tzeng *et al.*, 1990; Ser *et al.*, 2008).

Known values in literature on maximum allowable content of glucosinolate and phytic acid levels are from 1–4.4 $\mu\text{mol/g}$ feed and 0.5–1 g/100 g feed, respectively, depending on the fish species and growth stage (Spinelli *et al.*, 1983; Hossain *et al.*, 1991; Burel *et al.*, 2000; Burel *et al.*, 2001). Therefore, the contents of ANF in RPC are much less than the maximum allowable value concerning that the RPC makes only about 25–50% of the feed depending on fish species by replacement level of 100%. Only for sinapic acid no literature data were reported. However, the values detected in the RPC are minimal compared to the de-oiled meal (before the ethanolic extraction) with content of 15.2 g/kg sinapic acid and sinapic acid ester (SAE), 1.09 g/kg sinapic acid (SA), 9.03 g/kg sinapin (SIN) and 2.21 g/kg sinapoylglucose (SG). In view of solubility of these compounds in aqueous solution it suggests that the ethanolic extraction was able to remove the sinapic acid and its ester.

To summarize it can be said that from the nutritional point of view RPC can be compared with fish meal protein.

4.3 Fish meal replacement

The produced rapeseed protein concentrate was evaluated as fish meal replacement in diets for juvenile rainbow trout (*Oncorhynchus mykiss*), juvenile turbot (*Psetta maxima*), juvenile common carp (*Cyprinus carpio* L.) and juvenile wels catfish (*Silurus glanis* L.). The fish feeding trials are carried out by our project partner, GMA – Society/ Association for marine aquaculture Ltd. The results of the fish meal replacement study are published by Slawski (2011).

Experimental results from the conducted feeding trials demonstrate an enormous potential of RPC as protein source in aquafeeds. In the growth trial with rainbow trout 100% of

dietary fish meal was successfully replaced with RPC. Thirty-three percent of fish meal protein from a control diet could be substituted by RPC in diets of turbot and common carp without negative effects on feed efficiencies and fish growth. Wels catfish accepted substitution levels from 25%. The reason of the reduced fish meal replacement level by these fish species appears to be the unfavourable diet taste resulting in reduced diet intake and growth performances. Despite lower glucosinolate content the typical mustard smell of glucosinolates was still noticeable in the feed. This smell clearly adverse affected diet acceptance by these fish species.

Simple rapeseed and canola products (meal or cake) have been widely tested as protein sources in diets for several fish species. Different replacement levels of fish meal have been achieved. An overview is given by Enami (2011). A canola protein concentrate produced by the method developed by MCN Bioproducts Ltd. has been successfully incorporated into diets fed to rainbow trout and atlantic salmon (Drew, 2004; Drew *et al.*, 2007; Maenz, 2007a, 2007b; Thiessen *et al.*, 2004). Single aqueous protein extraction, de-phytization of extract by phytase treatment as well as precipitation of proteins followed by drying provided a canola protein concentrate (CPC) with slightly lower protein content (about 67%) and lower lysine and methionine and cysteine content, and completely no content of phytic acid relative to our RPC. However, the glucosinolate content (3.4 $\mu\text{mol/g}$) was significantly higher toward our RPC, which causes a reduction of diet palatability resulting in reduced feed intake and growth rates when CPC completely replaces fish meal. The CPC could replace up to 75% of fish meal protein in diet of rainbow trout and 50% in diet of atlantic salmon without adversely affecting growth or feed efficiency. Therefore, a complete replacement of fish meal by the canola protein concentrate produced by MCN process could not succeed. In contrast, our developed method provides a protein concentrate that successfully replaces 100% of fish meal in rainbow trout feeds.

5 Conclusions

The present study undertook investigations on the preparation of rapeseed proteins and developed manufacturing process of high quality rapeseed protein products, which can be used in fish nutrition.

The rapeseed meal produced in oil mills is thermally damaged. Consequently, the protein solubility (measured as PDI) is drastically reduced. An extraction of the proteins from the commercial meal is possible, but with low yields and reduced nutritive and functional protein properties. Protein extraction methods based on commercially available rapeseed meal would not be acceptable from an economic point of view.

Therefore, technological processes for the production of rapeseed protein concentrate, by starting from the seed and utilizing gentle meal processing and appropriate extraction procedure to get a protein product with high nutritive potential and high yield, were evaluated in this study. All process trials are conducted at the pilot scale, which means that a scale-up of the investigated production process from laboratory scale was already done compared to other methods described in literature.

The investigated rapeseed protein preparation process provides a rapeseed protein concentrate with high nutritional value and low value of antinutritive factors. In regard to well-balanced amino acid composition, it is appropriate for aquaculture feeds, which makes it very attractive as an alternative protein source in aquafeed.

All feeding tests demonstrate the great potential of RPC as fish feed ingredient in the nutrition of the investigated fish species. The highest fish meal replacement level (up to 100%) was observed in the feeding trials with rainbow trout. Therefore, especially in the nutrition of rainbow trout, RPC was identified as an excellent fish meal alternative. The achieved great results a completely replacement of fish meal by RPC obtained by the investigated production method, promise the best chance of its commercialization.

Acknowledgements. The authors would like to acknowledge the European Fisheries Fund (EFF) and the Future Programme for Fishery in Schleswig-Holstein for their financial support of the present research project.

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Cite this article as: Halime Nihat Adem, Ralf-Peter Tressel, Frank Pudel, Hanno Slawski, Carsten Schulz. Rapeseed use in aquaculture. OCL 2014, 21(1) D105.