Fractionation of rapeseed meal by milling, sieving and air classification – Effect on crude protein, amino acids and fiber content and digestibility

Jon Øvrum Hansen[1*], Anders Skrede[1], Liv Torunn Mydland[1], Margareth Øverland[1]

[1]Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway.

*Corresponding author [Telephone: +47-67232666; E-mail: jon.hansen@nmbu.no].

Abstract

Rapeseed meal (RSM), obtained as solvent extracted or expeller meal, is a feed commodity that is highly available. The high levels of fiber is a bottleneck for high inclusion in feed for monogastric farmed animals. In the present study, sieving and air classification were used to reduce fiber content in rapeseed products. The two first experiments unveiled the possibility to air classify rapeseed products with lipid content ranging from 20 to 160 g/kg, and to obtain fractions where crude protein (CP) content was increased from 325 to 376 g/kg and neutral detergent fiber (aNDFom) was reduced from 185 to 78 g/kg. Experiment 3 showed that ball milling of RSM in combination with sieving gave high separation of hulls and kernel. In the finest sieved fraction (0-150 µm), CP was increased from 336 (parent meal) to 394 g/kg with a fraction yield of 423 g/kg. Air classification of pre-sieved RSM had minor effect on CP and fiber levels, indicating a limited potential to further increase CP content when the hulls have partly been removed. Coefficient of total tract apparent digestibility (CTTAD) of CP, amino acids and lipids

in RSM fractions obtained with ball milling and sieving was determined in mink (*Neovison vison*). The average CTTAD for CP was higher ($P < 0.05$) in the high CP fine fraction (0.748) compared to the parent meal (0.702) and the coarse RSM fraction (0.635). In general, the CTTAD for amino acids followed the same trends as for CP, with significantly lowest digestibility for the coarse RSM fraction containing most hulls. The CTTAD of threonine and lysine was lowest among the essential amino acids, while cysteine had the lowest CTTAD among non-essential amino acids. To conclude, ball milling and sieving showed higher potential for fiber removal from RSM than ball milling and air classification. The reduced fiber content and increased CP content resulted in a higher digestibility of CP and amino acids.

Keywords: Rapeseed meal; Air classification; Fractionation; Nutrient digestibility; Amino acids; Mink.

Abbreviations: ADFom, ash corrected acid detergent fiber; CF, coarse fraction; CP, crude protein; CTTAD, coefficient of total tract apparent digestibility; FF, fine fraction; aNDFom, ash corrected neutral detergent fiber; RSM, rapeseed meal.

1. Introduction

Rapeseed or canola meal contains 320-400 g crude protein (CP)/kg and is a CP source that is highly available for animal feed producers. The amino acid profile is typically well balanced and high in sulfur containing amino acids (Newkirk et al., 2003). The high levels of anti-nutritional factors and fiber have been the main bottleneck limiting the inclusion of rapeseed meal (RSM) in feed for monogastric animals. The content of fiber can exceed 350 g/kg, and high

47 inclusion levels of RSM results in a major dilution of available energy in animal feed. The
48 content of hulls in whole rapeseed is about 140-180 g/kg and a main contributor to the total fiber
49 content in RSM (King and Dietz, 1987; Kracht et al., 2004). After oil extraction, the hull content
50 in RSM may be as high as 300-330 g/kg (Diosady et al., 1986; King and Dietz, 1987). The hulls
51 represent approximately 70% of the total lignin content in the RSM (Bell and Shires, 1982).
52 Removal of the hulls prior to oil extraction is rarely done due to loss of oil during dehulling and
53 difficulties with extracting oil from the dehulled rapeseed (Khajali and Slominski, 2012).
54 Research on upgrading RSM by partly removing hulls after oil extraction has been carried out by
55 use of sieving (Mustafa et al., 1996; Mejicanos, 2015), by a combination of sieving and air
56 classification (Diosady et al., 1986), or by use of air classification (King and Dietz, 1987; Zhou
57 et al., 2013; Zhou et al., 2015).
58 Air classification, a fractionation technique based on differences in shape and density of
59 particles, is a widely used method to separate CP (light fraction) from starch (heavy fraction) in
60 starch-rich cereals and legumes (Vose, 1978). This technique has low physical impact on the
61 particles and thus allows retaining of the native functionality of starch and CP. In addition, dry
62 fractionation such as air classification is often more energy efficient compared to wet-
63 fractionation techniques (Schutyser and van der Goot, 2011). Air classification has been used to
64 reduce fiber content in both soybean meal (Challa et al., 2010) and distillers dried grains with
65 solubles (Srinivasan et al., 2008), but is less applicable to high-fat materials like rapeseed due to
66 agglomeration and stickiness of particles. This problem may be aggravated if the soapstock
67 fraction, resulting from removal of free fatty acids from the oil, is added to the solvent extracted
68 RSM (dos Santos et al., 2014).

Another type of milling that has been tested for plant ingredients is low energy ball milling that is commonly used in the mineral and power industry (Takacs, 2002). In comparison with the jet mill, the ball mill have opposite criteria regarding intensity and energy usage, and this may create a different particle size distribution that can be an advantage for sieving or air classification.

It was hypothesized that milling equipment (ball mill vs. jet mill) and separation methods (sieving vs. air classification) might affect the yield and chemical composition of different RSM fractions. The objective of the present study was to investigate the possibility to separate the hull fraction from RSM by different mill types in combination with sieving or air classification. Mink (*Neovison vison*) was used as a model animal to determine coefficient of total tract apparent digestibility (CTTAD) of CP, amino acids and lipids in parent RSM and two rapeseed fractions with high or low fiber content.

2. Materials and methods

2.1 Preliminary experiments

2.1.1 Experiment 1

A Norwegian cold-pressed rapeseed cake (Askim Frukt- og Bærpresseri AS) with a lipid level of 221 g/kg was pretreated with 70% ethanol at 60°C for 30 min to partly remove lipids and sieved through a 80 µm mesh prior to jet milling at 3000 rpm and an air pressure of 5.2 bar (1.97 kg/h). The resulting rapeseed cake used for air classification contained 160 g lipid /kg. The jet mill used was a JMX-50 (Comex AS, Oslo, Norway) where the speed of the rotor determines the

92 particle size of the final product. The mill was equipped with a volumetric screw feeder (SFX-
93 30, Comex AS). The milled rapeseed cake was fractionated by an air classifier (ACX-50, Comex
94 AS, Oslo, Norway) at three different rotor speeds, 9000, 6000 and 4000 rpm, into a coarse and a
95 fine fraction. The air classifier was fitted with a SFX-30 screw feeder and a CX-100 cyclone
96 (Comex AS, Oslo, Norway). The coarse fraction from the first classification at 9000 rpm was
97 classified again at 6000 rpm. The coarse fraction from the 6000 rpm was further classified at
98 4000 rpm. The test was done without duplication, n=1.
99
100 *2.1.2 Experiment 2*
101 Commercial hexane-treated RSM with soapstock containing 33 g lipids/kg (Bunge,
102 Poland), was milled to an average particle size of 35 µm using a JMX-200 jet mill at 650 rpm
103 (51 kg/h). The meal was air classified using an ACX-200 classifier fitted with a CX-200 cyclone,
104 both delivered from Comex AS, Norway. The RSM was separated by multiple air classification
105 at three different rotor speeds; 2200, 1900, and 1700 rpm, into a coarse and a fine fraction. The
106 coarse fraction from the first classification at 2200 rpm was classified again at 1900 rpm. The
107 coarse fraction from the 1900 rpm classification was further classified at 1700 rpm. The test was
108 done without duplication, n=1.
109
110 *2.2. Experiment 3*
111 A commercial solvent extracted RSM without the soapstock fraction from oil refining,
112 containing 18 g lipid/kg, was purchased from ZT Kruszwica S.A., Poland. The RSM was milled
113 with a 5.56-L ball mill equipped with 4.5 kg 20 mm and 2 kg 40 mm steal balls for 1 or 2 h at 30
114 rpm and sieved through 150 and 300 µm sieves using a Haver & Boecker sieving machine (RX-

115 29-10, W.S.Tyler, OH, USA). The milling and sieving were performed with four individual
116 replicates, by using four randomly obtained samples from one big-bag of meal (n=4). The sieved
117 parts of 0-150 µm and 150-300 µm were fractionated by an air classifier in duplicate, n=2 (ACX-
118 50, Comex AS, Oslo, Norway). The fine part from the ball milling (0-150 µm) was classified
119 into a coarse and a fine fraction using 6000 and 9000 rpm rotor speed, whereas the coarse part
120 (150-300 µm) was classified at 500 and 1000 rpm (Fig.1).
121 The same batch of RSM was milled with a jet mill (JMX-50, Comex AS, Oslo, Norway)
122 at 1000 rpm and 2000 rpm, creating two batches of RSM with different particle size. The batch
123 produced by 2000 rpm jet milling was air classified using a rotor speed of 6000 and 9000 rpm,
124 whereas the batch from the 1000 rpm jet milling was air classified at 500 and 1000 rpm rotor
125 speed. The jet milling and classification was performed in duplicate, n=2. The air classifier was
126 fitted with a screw feeder and a CX-100 cyclone (Comex AS, Oslo, Norway).
127
128 *2.3. Digestibility experiment*
129 The mink trial was performed at the Norwegian University of Life Sciences. The
130 experimental procedures were approved by the Norwegian Animal Research Authorities and
131 were performed in accordance to the institutional and national guidelines for the care and use of
132 animals (the Norwegian Animal Welfare Act and the Norwegian Regulation and Animal
133 Experimentation).
134 The parent solvent extracted RSM meal (ZT Kruszwica S.A.) and two fractions similar to
135 the fractions obtained after ball milling and sieving in Experiment 3 were prepared to investigate
136 the *in vivo* CTTAD of CP, amino acids and lipids. The RSM was ball milled for 2 h and the
137 fractions obtained after sieving (0-150 µm and 150-300 µm) were tested (Table 1). The control

diet was based on fishmeal as the sole source of CP, and the test ingredients replaced 50% of the CP in the control diet (Table 2). Diets were prepared in advance, frozen at -22ºC in daily portions and placed in a refrigerator at 4ºC for thawing 24 h before feeding. In total 16 male minks (dark genotype, >6 months old) were randomly allocated to 4 dietary groups (n=4) and fed over a period of 7 d. The daily feed ration contained approximately 1.1MJ metabolizable energy, corresponding to the maintenance energy requirement in adult mink of 527 kJ/ kg $^{0.75}$ (Chwalibog et al., 1980). The feed was given as one meal per day and water was available *ad libitum*. The animals were kept in individual cages equipped for controlled feeding and quantitative fecal collection avoiding contamination with urine. Feces were collected every day during the last 4 d of the experimental period. After termination of the experiment, feces were weighed, freeze-dried, ground, and sifted for removal of hair before being analyzed. Digestibility was determined on individual basis and the average of four replicate animals, n=4.

2.4. Chemical and physical analyses

The RSM fractions, diets and fecal samples were analyzed for dry matter by drying to constant weight at 104°C (Commission dir. 71/393/EEC), and ash by incineration at 550°C (Commission dir. 71/250/EEC). CP was determined as Kjeldahl nitrogen × 6.25 (Commission dir. 93/28/EEC), crude lipid by Accelerated Solvent Extractor (ASE200, Dionex). Ash corrected amylase-treated neutral detergent fiber (aNDFom) was determined according to Mertens et al. (2002) using an Ankom 200 Fiber Analyzer with F58 Ankom filter bags. Determination of ash-corrected acid detergent fiber (ADFom) was done using the Ankom 200 Fiber Analyzer with F58 Ankom filter bags according to Method 973.18 (AOAC, 2000). Amino acids were analyzed

according to Commission dir. 98/64/EC on a Biochrom 30 amino acid analyzer (Biochrom Ltd., Cambridge, UK).

Particle size measurement was done by a Malvern RTSizer on-line spectrometer for dry particle determination. Pictures from RSM fractions in experiment 1 were obtained with an EVO 50 EP (Carl Zeiss AG, Oberkochen, Germany) scanning electron microscope.

2.5. Calculation and statistical analysis

The CTTAD was determined using collection of total feces for each individual animal as: (a-b)/a, where a is nutrient intake and b is amount of nutrient in feces. The CTTAD for CP and amino acids in the RSM products were obtained by the difference method, assuming that the CTTAD of CP and amino acids of fishmeal in diets with RSM products were equal to corresponding figures for the control diet (FM).

A complete randomized design using one-way analysis of variance (GLM) was used to differentiate among particle size distribution after ball milling and sieving, the obtained fraction yield, the content of CP, aNDFom, ADFom, and digestibility. Statistical significant ($P<0.05$) differences among diet means were ranked by Tukey's multiple range test and are indicated by different superscript letters in the tables. A 2*4 factorial approach was used for effects of milling and air classification on content of CP and aNDFom in RSM fractions. Yield, CP and aNDFom content were selected responses where time of milling, rotor speed during classification, sieved parts, and air classification fraction were the main effects for analyzing the results after ball milling. For analyzing the jet-milled samples, a similar 2*3 factorial design was used with the rpm of the jet mill, rotor speed during classification, and the given fractions after air

182 classification as main effects. All statistical analyzes were conducted with the software package
183 SAS System Release 8.3 (SAS, 1990).
184
185 **3. Results**
186
187 *3.1. Preliminary experiments*
188 The processing of the RSM in Experiment 1 and 2 unveiled problems with agglomeration
189 during jet milling and air classification. The stickiness of the particles resulted in agglomeration
190 of fine particles around the cone and the rotor in the cyclone, which lead to decreased output of
191 fines. The milling capacity in Experiment 1 was 1.97 kg/h.
192 The preliminary experiments were performed in one run without replications, so no
193 statistical evaluation was possible. Still, the data revealed a possibility to partly separate fractions
194 that differ in CP and fiber content (Fig. 2 and 3). The fine fraction contained more CP and less
195 aNDFom and ADFom than the coarse fraction after air classification. The reduction in ADFom
196 in the fine fractions was greater than corresponding figures for aNDFom in both experiments.
197 Color and shape of the fine and coarse fractions in Experiment 1 are shown in Fig. 4. The large
198 flat particles shown in Fig. 4D indicate that the coarse fraction contained intact pieces of hulls.
199
200 *3.2. Experiment 3*
201 Yields of the different sieved proportions after 1 and 2 h of ball milling differed
202 ($P<0.001$) (Table 3). The yield of the fine part (0-150 µm) was lower ($P<0.001$) for 1 h milling
203 than for 2 h milling. The CP and aNDFom contents of the fine part (0-150 µm) were similar for 1

and 2 h of milling, whereas the coarse part (>300 µm) from 2 h of milling had higher levels of aNDFom and ADFom, and lower CP levels compared to 1 h of milling.

The different sieved fractions from 1 and 2 h ball milling were difficult to separate with air classification at the given rotor speeds (Table 4). The yield of fine fraction ranged from 13 to 58 g/kg for the sieved fine part (0-150 µm) and from 42 to 154 g/kg for the sieved coarse part (150-300 µm). Time of milling, rotor speed during classification, fine vs. coarse fraction, and particle size, significantly contributed to the difference in CP level ($P<0.001$) of the ball-milled samples.

The aNDFom contents in the air classified samples were significantly affected by particle size and fine vs. coarse fraction, whereas milling time and rotor speed showed no effect. In general, fractionation gave an inverse relationship of the CP and aNDFom levels in which the fine fractions had a higher CP but a lower aNDFom level than the coarse fraction.

For the jet-milled material, no significant effects on CP and aNDFom levels were identified by the factorial analysis (Table 5). The jet milling at 1000 and 2000 rpm showed a capacity of 2.97 and 1.4 kg/h, respectively, and resulted in only minor differences in the CP of the fine and coarse fractions after air classification.

3.3 Chemical composition and digestibility

The proximate composition and amino acid profile of the fishmeal, parent RSM, and sieved RSM fractions tested in the digestibility experiment are presented in Table 1. The amino acid composition of the fractions of RSM was similar to the parent RSM. The content of lysine and methionine was lower than in the fishmeal protein, while the histidine content was higher.

Among non-essential amino acids, the content of cysteine, glutamine and proline was higher in RSM than in the fishmeal protein.

The CTTAD for CP was significantly lower for all RSM fractions compared to the fishmeal control, varying from 0.861 for fishmeal to 0.635 for the coarse RSM fraction containing most hulls (Table 6). The CTTAD of the individual amino acids was lower and more variable for parent meal and fractions of RSM than for fishmeal. The coarse RSM fraction revealed the lowest and most variable CTTAD for amino acids. Among the essential amino acids, threonine and lysine were the least digestible in the RSM fractions. Cysteine, asparagine and serine were typically poorly digestible non-essential amino acids in RSM. The CTTAD for crude lipid was significantly lower for the diet containing the coarse RSM fraction as compared to the other diets.

4. Discussion

Our preliminary fractionation experiments showed that it is possible to separate RSM into fractions differing in CP and fiber levels by air classification. These experiments also unveiled the possibility to jet mill and air classify rapeseed products varying in lipid content from 33 g/kg to 160 g/kg. High lipid content increases the plasticity of plant material which further increases agglomeration and reduces capacity during milling and air classification (Dijkink et al., 2007). Agglomeration of particles during milling and air classifying, was seen in all our experiments, indicating that other factors than lipid content, such as the water content (Tyler and Panchuk, 1982) and surface properties of the particles (Buckton, 1997), contribute to agglomeration during processing. Considering the high lipid level in the rapeseed cake used in Experiment 1 (160

249 g/kg), the results from milling and air classification were better than expected. This rapeseed
250 cake was treated with 70% ethanol prior to milling, which may have removed most of the polar
251 lipids and helped to reduce agglomeration. Despite the high lipid level, this rapeseed cake
252 behaved similar to the commercial hexane-treated RSM used in Experiment 2 in terms of the
253 different fractions with fairly similar particle size. In addition, the high-lipid RSM in Experiment
254 1, revealed an approximately twofold increase in milling capacity compared to the RSM without
255 the soapstock fraction used in Experiment 3. This could be due to changed particle surface
256 behavior caused by the ethanol treatment of the RSM used in Experiment 1. In previous studies,
257 it has been suggested that formation of surface ethoxy groups can prevent agglomeration (Smith
258 and Maskara, 1993).
259 A chemical process employing phosphoric acid followed with an alkaline solution is
260 commonly used to remove free fatty acids from raw plant based oils (O'Brien, 2008). This
261 soapstock fraction, is commercially often used as a feed additive and added to the solvent
262 extracted RSM. The soapstock contains mainly free fatty acids, but also lipid mixtures with
263 different polarity (Dumont and Narine, 2007). When the soapstock is mixed with the solvent
264 extracted RSM, the sticky consistency of the soapstock makes the meal clumpier and less
265 flowable, and the meal particles will most likely achieve a higher rate of plasticity. The authors
266 expected that the RSM without soapstock used in Experiment 3 would be less sticky and create
267 less agglomeration. However, the obtained results from fractionation indicate that the RSM
268 without soapstock did not differ considerably from the other types of RSM.
269 In air classification of peas, the shift between the “heavy” and “light” fractions occurs at
270 about 15-18 µm and pin mills are often used as milling equipment (Vose et al., 1976; Wu and
271 Nichols, 2005). Earlier experiments have shown that RSM milled and classified in a similar way

shows only a minor shift in CP content, but a somewhat greater shift in NDF content (King and Dietz, 1987; Zhou et al., 2015). This is probably due to similarities in weight and shape of the proteins and the different fiber fractions after milling. However, it has been reported that the coarse and heavy fraction contained more fiber, had a darker color, and contained more hulls than the light fraction (Seth and Clandinin, 1973; Diosady et al., 1986), which is in line with results from the present study. The change in color of the different RSM fractions, as seen in Fig. 3, shows that the level of black hulls from the RSM has increased in the coarse fraction. The pure RSM hulls contain about 150 g CP/kg (Kracht et al., 2004), and it is possible that the shift in CP is mostly due to the removal of hulls from the more kernel-rich fine fraction as discussed by King and Dietz (1987). This view may be supported by the rather poor shift in CP level after air classification of the sieved parts in Experiment 3, since the kernel and the hulls had already been partly separated. The fine fractions after air classification of sieved materials had actually lower CP and higher aNDFom levels than the parent meal, indicating limited possibility to further increase the level of CP after sieving with air classification.

The obtained increase in CP content in the sieved fine part after 2 h ball milling in our study is close to the theoretically maximum increase by removing the hull fraction. This increase in CP content combined with a reduction in ADFom and aNDFom in combination with the given part yields after 2 h ball milling and sieving is superior compared to previous results from milling and sieving of solvent extracted RSM (Mustafa et al., 1996; Mejicanos, 2015). One reason for this could be the use of ball milling in the present study instead of the traditionally used impact mills. Ball milling is a gentler and a lower energy consuming process that powders the yellow kernel and keeps the hulls as large and flaky particles that makes it possible to sieve the RSM into different fractions.

295 The CP and amino acid digestibility of LT fishmeal were within expected values and
296 according to previous experiments with mink (Vhile et al., 2005; Skrede et al., 2011). The low
297 digestibility of cysteine in all diets is in agreement with other digestibility experiments with mink
298 where various ingredients have been tested (Skrede et al., 1998; Vhile et al., 2005; Skrede et al.,
299 2011).
300 In the present study, digestibility was determined with the carnivorous mink as a model
301 animal, since apparent amino acid digestibility in mink has been shown to be highly correlated
302 with ileal digestibility in pigs (Skrede et al., 1998). The standard ileal digestibility in pigs of
303 solvent and expeller extracted RSM was recently reviewed by Mejicanos et al. (2016). They
304 showed that the amino acid digestibility varied among experiments, but the ileal digestibility of
305 the individual amino acids followed the same trend as the CTTAD of the parent RSM in the
306 present study. Experiments have shown that the ileal digestibility of amino acids is higher in pigs
307 fed dehulled RSM compared to meal without dehulling (Grala et al., 1998), and that addition of
308 100 g/kg hulls from black rapeseed decreases ileal amino acid digestibility in pigs (Mitaru et al.,
309 1984). The latter is in line with differences in CTTAD among the RSM fractions in the present
310 study, where the hull-rich fraction showed significantly lower amino acid digestibility than the
311 parent meal and the fine fraction. Mitaru et al. (1984) suggested that the reduction in CP
312 digestibility was linked to the high lignin content in the hull fiber. They also indicated that the
313 high levels of tannins could interfere with proteases and thereby reduce CP digestibility as
314 previously described (Reddy et al., 1985). The decrease in digestibility with increased level of
315 hulls in the present study could be partly due to low digestibility of protein located in the hulls.
316 Hulls contain approximately 150 g CP/kg, and oil extracted RSM contains 300-330 g hulls/kg
317 (Diosady et al., 1986). The hulls have approximately 260 g lignin/kg, which may create a strong

intercellular skeleton that prevents the action of digestive enzymes (Knudsen, 2014) and reduce digestibility of protein and amino acids present in the hull matrix.

In conclusion, air classification is a fractionation technique suitable for obtaining RSM fractions varying in CP and fiber content of meal with different lipid content. Jet milling and air classification is, however, techniques that require high-energy input during milling and classification, and the results from this study indicate that low energy input methods such as ball milling, followed by sieving can give higher CP fraction yield and better up-concentration and removal of fiber compared to air classification. The high-protein fraction had a higher amino acid digestibility than the high-fiber RSM fraction, but not compared to the parent meal. Further research on ball milling and sieving are needed to maximize CP yield and fiber removal from RSM for improved nutritional value and use in farm animal diets.

5. Acknowledgements

The research was supported by FeedMileage - Efficient use of feed resources for a sustainable Norwegian food production (the Research Council of Norway; grant no. 233685/E50). Considerable thanks goes to Jacek Kolacz and Marcin Jarosz in Comex AS for their expertice during air classification and discussion of results.

6. References

AOAC International. 2000. Official methods of analysis of AOAC International. 17th edition. Gaithersburg, MD, USA, Association of Analytical Communities.

Bell, J.M., Shires, A., 1982. Composition and digestibility by pigs of hull fractions from rapeseed cultivars with yellow or brown seed coats. Can. J. Anim. Sci. 62**,** 557-565.

342 Buckton, G., 1997. Characterisation of small changes in the physical properties of powders of
343 significance for dry powder inhaler formulations. Adv. Drug Del. Rev. 26**,** 17-27.
344 Challa, R., Srinivasan, R., To, F., 2010. Fractionation of soybean meal, cottonseed meal and
345 wheat middlings using combination of sieving and air classification. Anim. Feed Sci.
346 Technol. 159**,** 72-78.
347 Chwalibog, A., Glem-Hansen, N., Henckel, S. Thorbek, G. 1980. Energy metabolism in adult
348 mink in relation to protein-energy levels and environmental temperature. Proc. 8th Symp.
349 on Energy Metabolism. EAAP Publ. No. 26, 283-286.
350 dos Santos, R.R., Muruci, L.N.M., Santos, L.O., Antoniassi, R., Silva, J.P.L.d., Damaso, M.C.T.,
351 2014. Characterization of different oil soapstocks and their application in the lipase
352 production by *Aspergillus niger* under solid state fermentation. J. Food Nutr. Res. 2**,** 561-
353 566.
354 Dijkink, B.H., Speranza, L., Paltsidis, D., Vereijken, J.M., 2007. Air dispersion of starch-protein
355 mixtures: A predictive tool for air classification performance. Powder Technol. 172**,** 113-
356 119.
357 Diosady, L.L., Rubin, L.J., Tar, C.G., Etkin, B., 1986. Air classification of rapeseed meal using
358 the tervel separator. Can. J. Chem. Eng. 64**,** 768-774.
359 Dumont, M.-J., Narine, S.S., 2007. Soapstock and deodorizer distillates from North American
360 vegetable oils: Review on their characterization, extraction and utilization. Food Res. Int.
361 40**,** 957-974.
362 Grala, W., Verstegen, M.W., Jansman, A.J., Huisman, J., van Leeusen, P., 1998. Ileal apparent
363 protein and amino acid digestibilities and endogenous nitrogen losses in pigs fed soybean
364 and rapeseed products. J. Anim. Sci. 76**,** 557-568.

365 Khajali, F., Slominski, B.A., 2012. Factors that affect the nutritive value of canola meal for
366 poultry. Poult. Sci. 91**,** 2564-2575.
367 King, R.D., Dietz, H.M., 1987. Air classification of rapeseed meal. Cereal Chem. 64**,** 411-413.
368 Knudsen, K.E.B., 2014. Fiber and nonstarch polysaccharide content and variation in common
369 crops used in broiler diets. Poult. Sci. 93**,** 1-14.
370 Kracht, W., Dänicke, S., Kluge, H., Keller, K., Matzke, W., Hennig, U., Schumann, W., 2004.
371 Effect of dehulling of rapeseed on feed value and nutrient digestibility of rape products in
372 pigs. Arch. Anim. Nutr. 58**,** 389-404.
373 Mejicanos, G., Sanjayan, N., Kim, I.H., Nyachoti, C.M., 2016. Recent advances in canola meal
374 utilization in swine nutrition. J. Anim. Sci. Technol. 58**,** 7.
375 Mejicanos, G.A., 2015. Tail-end dehulling of canola meal: chemical composition and nutritive
376 value of dehulled meal for broiler chickens and weaned pigs. Master Thesis. University
377 of Manitoba. USA.
378 Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in
379 feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Intern. 85**,**
380 1217-1240.
381 Mitaru, B.N., Blair, R., Reichert, R.D., Roe, W.E., 1984. Dark and yellow rapeseed hulls,
382 soybean hulls and a purified fiber source: Their effects on dry matter, energy, protein and
383 amino acid digestibilities in cannulated pigs. J. Anim. Sci. 59**,** 1510-1518.
384 Mustafa, A.F., Christensen, D.A., McKinnon, J.J., 1996. Chemical characterization and nutrient
385 availability of high and low fiber canola meal. Can. J. Anim. Sci. 76**,** 579-586.
386 Newkirk, R.W., Classen, H.L., Scott, T., A. Edney, M.J., 2003. The digestibility and content of
387 amino acids in toasted and non-toasted canola meals. Can. J. Anim. Sci. 83**,** 131-139.

O'Brien, R.D., 2008. Fats and oils: Formulating and processing for applications, 3nd ed., CRC Press, Boca Raton, New York.

Reddy, N.R., Pierson, M.D., Sathe, S.K., Salunkhe, D.K., 1985. Dry bean tannins: A review of nutritional implications. J. Am. Oil Chem. Soc. 62**,** 541-549.

SAS 1990. Statistical Analysis System, User`s guide. Version 6, 4th ed., SAS institute, Cary, NC, USA.

Schutyser, M.A.I., van der Goot, A. J., 2011. The potential of dry fractionation processes for sustainable plant protein production. Trends Food Sci. Technol. 22**,** 154-164.

Seth, P.C.C., Clandinin, D.R., 1973. Metabolisable energy value and composition of rapeseed meal and of fractions derived therefrom by air-classification. Br. Poult. Sci. 14**,** 499-505.

Skrede, A., Berge, G., Storebakken, T., Herstad, O., Aarstad, K., Sundstøl, F., 1998. Digestibility of bacterial protein grown on natural gas in mink, pigs, chicken and Atlantic salmon. Anim. Feed Sci. Technol. 76**,** 103-116.

Skrede, A., Mydland, L., Ahlstrøm, Ø., Reitan, K., Gislerød, H., Øverland, M., 2011. Evaluation of microalgae as sources of digestible nutrients for monogastric animals. J. Anim. Feed Sci. 20, 131-142.

Smith, D.M., Maskara, A., 1993. Preparation of well dispersed systems: The role of oxide bridging in agglomerate formation. Intern. Fine Particle Res. Inst. 1**,** 251-268.

Srinivasan, R., Yadav, M.P., Belyea, R.L., Rausch, K.D., Pruiett, L.E., Johnston, D.B., Tumbleson, M.E., Singh, V., 2008. Fiber separation from distillers dried grains with solubles using a larger elutriation apparatus and use of fiber as a feedstock for corn fiber gum production. Biol. Eng. 1**,** 39-49.

Takacs, L. 2002. Self-sustaining reactions induced by ball milling. Prog. Mater Sci. 47**,** 355-414.

411 Tyler, R.T., Panchuk, B.D., 1982. Effect of seed moisture content on the air classification of field
412 peas and faba beans. Cereal Chem. 59, 31-33.
413 Vhile, S.G., Skrede, A., Ahlstrøm, Ø., Hove, K., 2005. Comparative apparent total tract
414 digestibility of major nutrients and amino acids in dogs (*Canis familiaris*), blue foxes
415 (*Alopex lagopus*) and mink (*Mustela vison*). Anim. Sci. 81**,** 141-148.
416 Vose, J.R. 1978., Separating grain components by air classification. Separ. Purif. Rev. 7**,** 1-29.
417 Vose, J.R., Basterrechea, M.J., Gorin, P.A.J., Finlayson, A.J., Youngs, C.G., 1976. Air
418 classification of field peas and horsebean flours: chemical studies of starch and protein
419 fractions. Cereal Chem. 53**,** 928-936.
420 Wu, Y.V., Nichols, N.N., 2005. Fine grinding and air classification of field pea. Cereal Chem.
421 82**,** 341-344.
422 Zhou, X., Oryschak, M.A., Zijlstra, R.T., Beltranena, E., 2013. Effects of feeding high- and low-
423 fibre fractions of air-classified, solvent-extracted canola meal on diet nutrient digestibility
424 and growth performance of weaned pigs. Anim. Feed Sci. Technol. 179**,** 112-120.
425 Zhou, X., Zijlstra, R.T., Beltranena, E., 2015. Nutrient digestibility of solvent-extracted *Brassica*
426 *napus* and *Brassica juncea* canola meals and their air-classified fractions fed to ileal-
427 cannulated grower pigs. J. Anim. Sci. 93**,** 217-228.

428 Table 1. Chemical composition, presented as-is of fishmeal (FM), parent rapeseed meal (RSM),
429 fine fraction (0-150 µm) and coarse fraction (150-300 µm) after 2 h ball milling and sieving,
430 used in the experimental diets.

	FM	Parent RSM	Fine fraction	Coarse fraction
Composition, g/kg				
Dry matter	925	890	885	900
Crude protein	685	336	398	313
Crude lipid	92	18	25	16
Ash	144	70	70	66
aNDFom		253	189	294
Amino acids, g/16 g N				
Total amino acids[1]	90.0	93.8	92.9	91.7
Essential amino acids				
Lysine	7.9	5.7	5.7	5.8
Threonine	4.3	4.8	4.6	4.7
Methionine	3.1	2.4	2.4	2.3
Valine	4.7	5.3	5.1	5.3
Isoleucine	4.0	4.2	4.2	4.1
Leucine	7.4	7.4	7.4	7.1
Phenylalanine	3.7	4.0	4.1	4.0
Histidine	2.1	2.9	2.9	2.8
Arginine	6.3	6.0	6.1	5.7
Non-essential amino acids				
Cysteine	1.2	2.6	2.6	2.6
Asparagine	9.5	8.1	8.0	7.9
Serine	4.1	4.4	4.4	4.3
Glutamine	12.9	16.9	17.1	16.4
Proline	4.0	6.7	5.9	6.5
Glycine	6.4	5.3	5.3	5.1
Alanine	6.1	4.5	4.5	4.3
Tyrosine	2.5	2.7	2.7	2.8

431 [1] Total sum of amino acids without tryptophan.

432

433 Table 2. Formulation (as-is) and chemical composition of the experimental diets.

Diet	Control	Parent RSM	Fine fraction	Coarse fraction
Formulation, g/kg				
Fishmeal, LT[a]	510	255	255	255
Parent RSM[b]		513		
Fine fraction			434	
Coarse fraction				547
Wheat starch[c]	232	84	143	50
Soy oil[d]	145	145	145	145
Cellulose[e]	110		20	
Vitamin and mineral mix[f]	3	3	3	3
Chemical composition, g/kg				
Dry matter (DM)[g]	325	370	337	390
In DM				
Crude protein	358	374	360	355
Crude lipid	167	188	183	181
Ash	80	73	83	77
aNDFom	99	130	100	175

434 [a]Norsildmel, Egersund, Norway. [b]Rapeseed meal, solvent extracted, ZT Kruszwica S.A., Poland.
435 [c]Pregeflo®, Roquette Freres, Lestrem, France. [d]Soy oil, Food grade, Europris AS, Fredrikstad, Norway.
436 [e]Arbocel, BWW40, J. Rettenmaier & Söhne, Rosenberg, Germany. [f]Premix fur animals, Normin AS,
437 Hønefoss, Norway. Per kg feed ; Retinol 6000.0 IU, Cholecalciferol 600.0 IU, α-tocopherol SD 150 mg,
438 Thiamin 45 mg, Riboflavin 9 mg, d-Ca-Pantothenate 10 mg, Niacin 15 mg, Biotin 0.09 mg,
439 Cyanocobalamin 0.06 mg, Folic acid 11 mg, Pyridoxine 9 mg, Cu: Cu-sulfate $5H_2O$ 3.75 mg, Zn: Zinc
440 oxide 30.0 mg, Mn: Manganese oxide 22.5 mg, I: K-Iodide 0.19 mg, Ca 0.6 g. [g] Water was added to
441 suitable diet consistency.
442

443

444 Table 3. Particle size distribution, crude protein (CP), ash corrected neutral detergent fiber

445 (aNDFom) and ash corrected acid detergent fiber (ADFom) contents of solvent extracted

446 rapeseed meal milled for 1 and 2 h in a ball mill and the sieved fractions.

		1 h milling			2 h milling			s.e.m.[1]	P-value
g/kg	Parent meal	0-150µm	150-300µm	>300µm	0-150µm	150-300µm	>300µm		
Yield		228 [c]	325 [b]	446 [a]	423 [a]	357 [b]	221 [c]	2.8	<0.001
CP	336	402 [a]	367 [b]	292 [d]	394 [a]	331 [c]	237 [e]	6.2	<0.001
aNDFom	251	155 [e]	202 [d]	322 [b]	168 [e]	260 [c]	396 [a]	8.5	<0.001
ADFom	166	73 [e]	120 [d]	234 [b]	85 [e]	175 [c]	307 [a]	8.5	<0.001

447 [1] Pooled standard error of means, as-is. Different letter denote significant (P<0.05) difference between

448 rapeseed meal fractions. n = 4 replicates per treatment.

449

Table 4. Fraction yield and content of crude protein (CP) and ash corrected neutral detergent fiber (aNDFom) in air classified fine and coarse rapeseed meal fractions. Solvent extracted rapeseed meal was ball milled for either 1 or 2 h, sieved into 2 parts (0-150 µm and 150-300 µm) and thereafter air classified with high and low rotor speed, resulting in 16 different RSM fractions in a 2*4 factorial design (see overview of the experimental setup in Fig. 1).

		Air classification			
	Ball milling	Rotor speed	Fraction	0-150 µm	150-300 µm
Fraction yield (g/kg)					
	1 hour	High	Fine	13 ± 1	42 ± 1
			Coarse	987 ± 1	958 ± 1
		Low	Fine	17 ± 8	128 ± 5
			Coarse	983 ± 8	872 ± 5
	2 hour	High	Fine	27 ± 2	74 ± 13
			Coarse	973 ± 2	926 ± 13
		Low	Fine	58 ± 14	154 ± 1
			Coarse	942 ± 14	846 ± 1
CP (g/kg)					
	1 hour	High	Fine	346 ± 5	330 ± 1
			Coarse	401 ± 3	372 ± 5
		Low	Fine	379 ± 6	344 ± 1
			Coarse	400 ± 1	365 ± 4
	2 hour	High	Fine	323 ± 7	283 ± 7
			Coarse	389 ± 1	332 ± 1
		Low	Fine	371 ± 1	310 ± 2
			Coarse	394 ± 10	327 ± 5
aNDFom (g/kg)					
	1 hour	High	Fine	179 ± 11	252 ± 8
			Coarse	163 ± 1	197 ± 6
		Low	Fine	209 ± 22	236 ± 1
			Coarse	164 ± 4	211 ± 7
	2 hour	High	Fine	160 ± 34	295 ± 11
			Coarse	175 ± 1	258 ± 5
		Low	Fine	220 ± 9	264 ± 11
			Coarse	176 ± 5	187 ± 7

	P-values for the given factors				
	Model	Milling	Rotor speed	Fraction	Particle size
CP	<0.001	<0.001	<0.001	<0.001	<0.001
aNDFom	<0.001	0.0695	0.85	<0.001	<0.001

455 Values are given as mean, as-is (n = 2) ± standard deviation

456

457

458 Table 5. Fraction yield, crude protein (CP), and ash corrected neutral detergent fiber (aNDFom)
459 content in fine and coarse rapeseed meal fractions obtained after grinding at 2 different speeds in
460 the jet mill and air classification at high and low rotor speed in a 2*3 factorial design.

		Air classification		
	Jet milling	Rotor speed	Fraction	
Fraction yield (g/kg)				
	1000 rpm	High	Fine	214 ± 6
			Coarse	786 ± 6
		Low	Fine	262 ± 7
			Coarse	738 ± 7
	2000 rpm	High	Fine	52 ± 2
			Coarse	948 ± 2
		Low	Fine	81 ± 1
			Coarse	919 ± 1
CP (g/kg)				
	1000 rpm	High	Fine	349 ± 8
			Coarse	323 ± 2
		Low	Fine	342 ± 10
			Coarse	334 ± 11
	2000 rpm	High	Fine	356 ± 1
			Coarse	322 ± 2
		Low	Fine	362 ± 1
			Coarse	331 ± 20
aNDFom (g/kg)				
	1000 rpm	High	Fine	267 ± 7
			Coarse	307 ± 8
		Low	Fine	273 ± 8
			Coarse	275 ± 9
	2000 rpm	High	Fine	223 ± 34
			Coarse	288 ± 1
		Low	Fine	253 ± 47
			Coarse	294 ± 6

	P-value for given factorial factors			
	Model	Milling	Rotor speed	Fraction
CP	0.020	0.306	0.328	0.121
aNDFom	0.083	0.850	0.171	0.202

461 Values are given as mean, as-is (n = 2) ± standard deviation

462

463 Table 6**.** Coefficients of total tract apparent digestibility (CTTAD) of main nutrients and amino
464 acids in fishmeal (FM), parent rapeseed meal (PRSM), fine fraction (FF) (0-150 µm) and coarse
465 fraction (CF) (150-300 µm) after 2 h ball milling and sieving used in experimental mink diets.

	FM	PRSM	FF	CF	s.e.m.[1]	P-value [2]
Crude lipids [3]	0.990 [a]	0.989 [a]	0.988 [a]	0.982 [b]	0.002	0.001
Crude protein	0.861[a]	0.702[c]	0.747[b]	0.635[d]	0.018	<0.001
Amino acids						
Total amino acids	0.885 [a]	0.779 [b]	0.811 [b]	0.686 [c]	0.022	<0.001
Essential amino acids						
Lysine	0.927 [a]	0.746 [c]	0.790 [b]	0.642 [d]	0.018	<0.001
Threonine	0.819 [a]	0.662 [b]	0.683 [b]	0.538 [c]	0.050	<0.001
Methionine	0.916 [a]	0.846 [b]	0.852 [b]	0.727 [c]	0.024	<0.001
Valine	0.894 [a]	0.763 [b]	0.799 [b]	0.692 [c]	0.026	<0.001
Isoleucine	0.908 [a]	0.777 [bc]	0.826 [b]	0.712 [c]	0.031	<0.001
Leucine	0.922 [a]	0.840 [b]	0.857 [b]	0.774 [c]	0.027	<0.001
Phenylalanine	0.878 [a]	0.852 [a]	0.881 [a]	0.774 [b]	0.022	<0.001
Histidine	0.876 [a]	0.839 [a]	0.841 [a]	0.760 [b]	0.024	<0.001
Arginine	0.932 [a]	0.881 [b]	0.896 [b]	0.810 [c]	0.013	<0.001
Non-essential amino acids						
Cysteine	0.729 [a]	0.657 [a]	0.689 [a]	0.540 [b]	0.044	<0.001
Asparagine	0.811 [a]	0.694 [b]	0.730 [b]	0.574 [c]	0.027	<0.001
Serine	0.841 [a]	0.699 [b]	0.738 [b]	0.585 [c]	0.038	<0.001
Glutamine	0.908 [a]	0.852 [c]	0.879 [b]	0.806 [d]	0.013	<0.001
Proline	0.869 [a]	0.741 [b]	0.779 [b]	0.620 [c]	0.021	<0.001
Glycine	0.867 [a]	0.752 [b]	0.785 [b]	0.676 [c]	0.027	<0.001
Alanine	0.903 [a]	0.806 [b]	0.822 [b]	0.744 [c]	0.024	<0.001
Tyrosine	0.897 [a]	0.793 [b]	0.858 [a]	0.690 [c]	0.022	<0.001

466 [1]Pooled standard error of mean. [2] Different letters denote significant (P<0.05) difference among diets. n =
467 4 replicates per treatment. [3] Digestibility of crude lipid was calculated for the complete feed, not on
468 ingredient level.

469
470
471

472

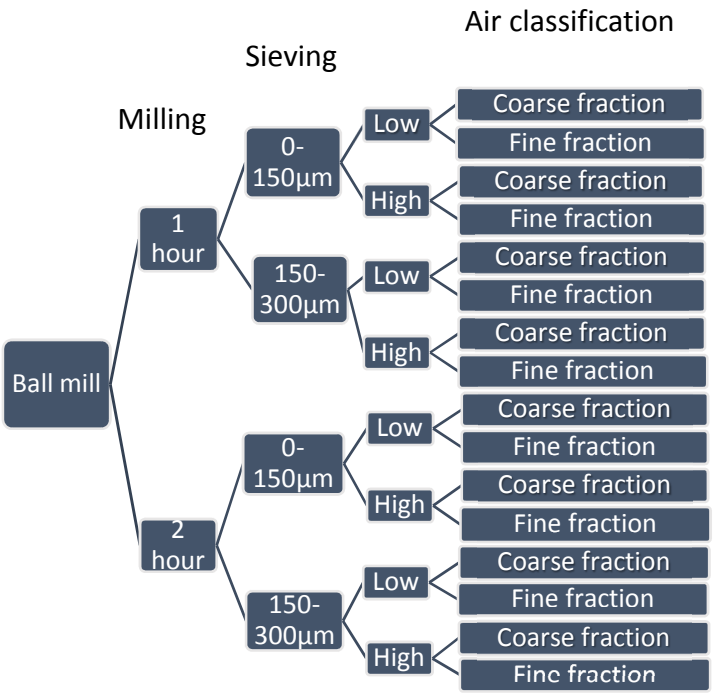

Fig. 1. Overview of the experimental setup for ball milling (1 or 2 h), sieving into smaller (0-150 µm) and larger particles (150-300 µm), and air classification at high or low rotor speed in Experiment 3.

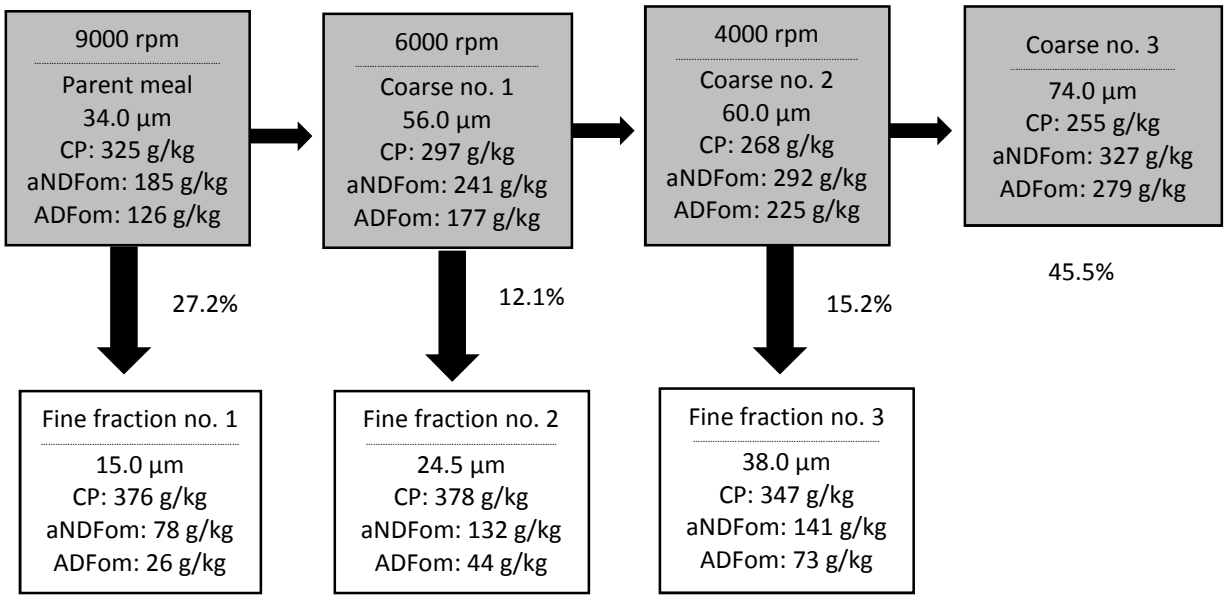

Fig. 2. Experiment 1. Yields, content of crude protein (CP), ash corrected neutral detergent fiber (aNDFom), ash corrected acid detergent fiber (ADFom), and particle size of fine and coarse fractions after multiple air classification of a high lipid cold pressed RSM (160 g lipid /kg) at 9000, 6000, and 4000 rpm rotor speeds in the air classifier.

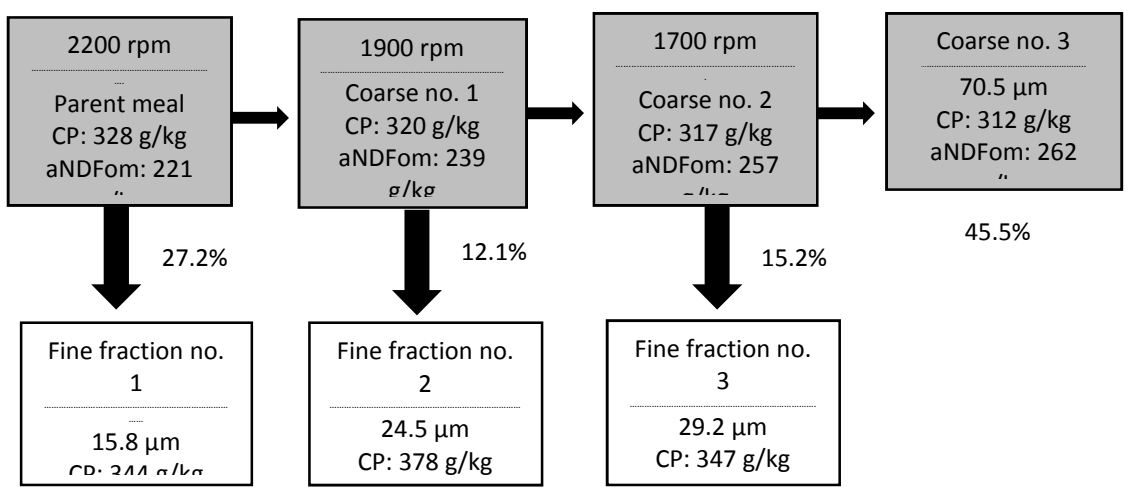

Fig 3. Experiment 2. Yields, content of crude protein (CP), ash corrected neutral detergent fiber (aNDFom), ash corrected acid detergent fiber (ADFom), and particle size of fine and coarse fractions after multiple air classification of a solvent extracted RSM (33 g lipid/kg) at 2200, 1900, and 1700 rpm rotor speeds in the air classifier.

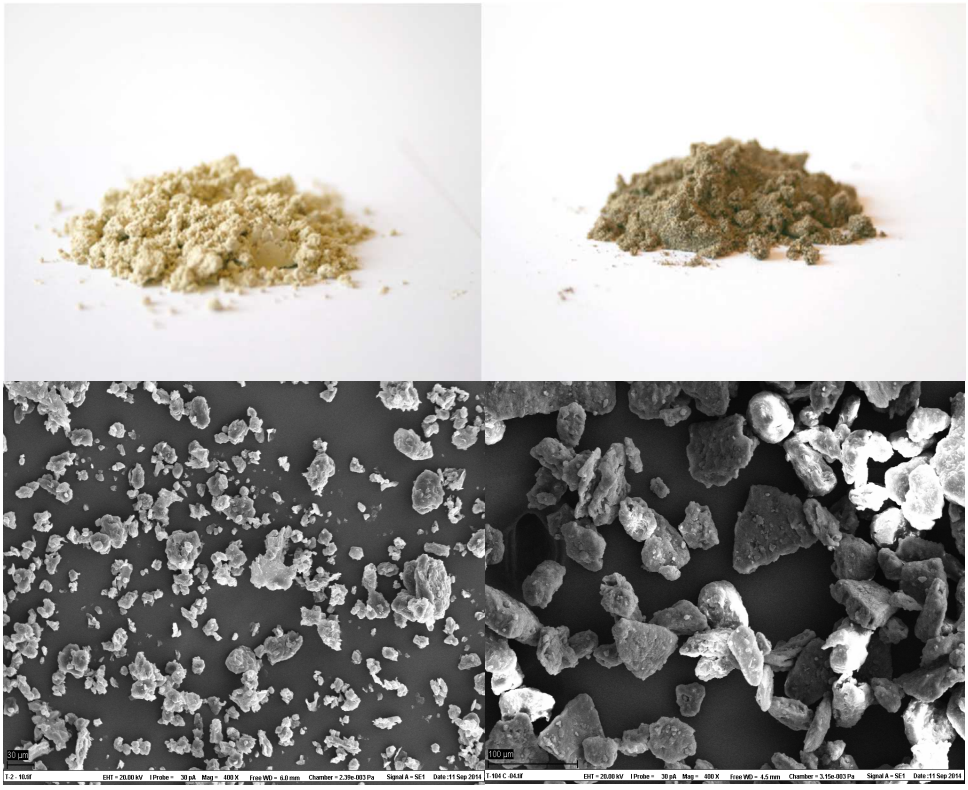

Fig. 4. Gross (A) and scanning electron microscopic (C) pictures of fine fraction no 1 and gross (B) and microscopic (D) pictures of the coarse fraction no 3 from Experiment 1.