Available online at www.sciencedirect.com

ScienceDirect

Agriculture and Agricultural Science Procedia 00 (2016) 000–000

Agriculture and Agricultural Science Procedia

www.elsevier.com/locate/procedia

5th International Conference "Agriculture for Life, Life for Agriculture"

Biochemical characterization of industrially produced rapeseed meal as a protein source in food industry

Petya Ivanova[a], Vesela Chalova[a]*, Galina Uzunova[a], Lidia Koleva[a], Ivan Manolov[b]

[a]*University of Food Technologies, 26 Maritsa Blvd, Plovdiv 4002, Bulgaria*
[b]*Agricultural University, 12 Mendeleev Str., Plovdiv 4000, Bulgaria*

Abstract

Rapeseed meal is a by-product of oil production which is primarily used in feed industry. The application of the rapeseed meal as a protein source in food industry is an alternative which leads to a better and more complete use of this by-product. Biochemical characteristics of industrially produced rapeseed meal vary and therefore, detailed analyses prior to its use as a protein source is necessary. The commercial rapeseed meal evaluated in this study contained high protein amount (39.86%) and low residual total fats (2.30%). It was characterized with low levels of glucosinolates (12.69 ± 0.18 µmol/g) and phenols (1.13 ± 0.04%). Amino acid analysis revealed lysine as the first limiting amino acid with an amino acid score of 58.00%, followed by valine (66.86%). However, this by-product was rich in leucine and isoleucine which amino acid scores equaled to 97.60 and 88.67% respectively. The amino acid score evaluation demonstrated relatively high amount of sulphur containing amino acids (82.57%). The commercial rapeseed meal exhibited low *in vitro* digestibility (18.59 ± 0.98%). The albumin, globulin and glutelin fractions however, expressed higher digestibility with albumin fraction being the most susceptible (67.22 ± 1.28%) to pepsin and pancreatin proteolytic activities.

Peer-review under responsibility of the University of Agronomic Sciences and Veterinary Medicine Bucharest © 2016 The Authors. Published by Elsevier B.V.
Keywords: rapeseed meal; biochemical characterization; digestibility.

Kommentar [S1]: Elsev with copyright entity, year, company Elsevier Ltd./B.V. conference organizer name.

* Corresponding author. Tel.: +359-32-603-855; fax: +359-32-644-102.
E-mail address: veselachalova@gmail.com

1. Introduction

Rapeseed is a major oil-bearing crop. From 1992 to 2012, the worldwide production of rapeseeds exceeded the one of the cottonseeds thus turning it to the second most important world's oilseed plant after soybean (Carré and Pouzet, 2014). During that period of time, China, India, Canada and European Union (27) contributed the most to the expansion of the rapeseed production. In Bulgaria, rapeseed is the second most cultivated oil-bearing crop after sunflower. In 2014, the surface area sown with rapeseed reached 191 572 ha, while seed production equaled 527 912 tons (Ministry of Agriculture and Food, Republic of Bulgaria, 2015). After the enhancement of rapeseed oil utilization as a feedstock for biodiesel generation, the worldwide production of rapeseeds has been predicted to increase (Carré and Pouzet, 2014).

Due to the high oil content (42%), rapeseeds are mainly used for oil production. They also contain relatively high amount of proteins (22-24%) which may increase up to 40% after oil extraction. The remaining product, known as rapeseed meal, represents approximately 48% of the total quantity of the rapeseeds used for oil production (Ivanova, 2012). Rapeseed meal is characterized with a relatively balanced amino acid composition with an except for lysine which makes it appropriate as a protein-rich additive in feed industry (Newkirk, 2009). However, its application as a feed ingredient is limited by the presence of anti-nutrients and high fiber content which impact protein digestibility and overall animal metabolisms. As a result, a considerable amount of the annual rapeseed meal remains unutilized thus converting into a rather waste product.

Other than utilized for feed supplementation, the rapeseed meal has the potential to serve as an alternative plant protein source for human consumption (Tan et al., 2011). However, the quality of rapeseed meal is highly variable and is dependent on various factors (Bell and Jeffers, 1976; Li et al., 2015). The type of rapeseed cultivar, environmental conditions during growth as well as soil type and composition affect the quality of the seeds which in turn, determines the quality characteristics of manufactured products (Bellostas Muguerza et al., 2007). Some alterations in rapeseeds properties during storage may also occur. The industrial procedure of oil extraction used as well as the seed pre-treatment may exert variations in anti-nutrient concentrations, protein digestibility and amino acid composition of rapeseed meal (Newkirk and Classen, 2002; Ayton, 2014). Therefore, a biochemical characterization of industrially produced rapeseed meal prior to its application as a protein source in food industry is necessary. Furthermore, while published data obtained on rapeseed meal produced and analyzed under laboratory conditions are abundant, studies performed on industrially produced rapeseed meal are scarce. The goal of the current study was to establish the biochemical characteristics of rapeseed meal, industrially produced in Bulgaria. The amino acid composition as well as in vitro rapeseed meal protein digestibility was also evaluated.

2. Material and methods

2.1. Material

Rapeseed meal was provided by a local company. It was produced after thermal treatment of rapeseeds at 110-115ºC and oil extraction with hexane at 60-65ºC. The industrially produced rapeseed meal was additionally grinded and sifted to collect 0.315 mm particles which were used for analyses. All reagents used were of analytical grade.

2.2. *Biochemical characterization of rapeseed meal*

Total nitrogen was determined by Kjeldal's method and multiplied by 6.25 to convert to crude protein (Tomov et al., 2009). Biuret method (AACC, 1983) was used to evaluate protein content in extracts whenever it was needed. Bovine serum albumin was used for standard curve generation. Crude fat content of the rapeseed meal was determined by Soxhlet extraction method (ISO 7302:2003). Fiber and ash contents were determined by ICC Standard №156 and ICC Standard №104/1 respectively. Total phenols were extracted as describe by Villanueva et al. (1999) and quantified by using Folin-Ciocalteu reagent (Ainsworth and Gillespie, 2007). Total glucosinolates were evaluated as described by Jezek et al. (1999). The method is based on spectrophotometric evaluation of glucosinolates after alkaline hydrolysis and reduction with potassium ferricyanide. Sinigrin was used for standard curve generation.

2.3. Amino acid analysis and amino acid score calculation

Amino acids were analyzed by using Gas Chromatography–Mass Spectrometry (GC-MS) (Agrobioinstitute, Sofia, Bulgaria). The sample was hydrolyzed with 6N HCl and lyophilized. The amino acids were derivatized by silylation and evaluated by using a gas chromatograph Agilent GC 7890 and a mass-spectral detector Agilent MD 5975 (Agilent Technologies, Inc., Wilmington, DE). NIST'08 (National Institute of Standards and Technology, USA) and the Golm Metabolome Databases (http://csbdb.mpimp-golm.mpg.de/csbdb/gmd/gmd.html) were used to identify the amino acids in rapeseed meal. Amino acid score was calculated as a ratio of the amount of each essential amino acid determined in the rapeseed meal (g/100g protein) and the amount of the respective amino acid in an "ideal" protein (g/100g protein) as stated by the Food and Agriculture Organization of the United Nations (FAO, 1970).

2.4. Digestibility of rapeseed meal protein and rapeseed meal protein fractions

Albumin, globulin and glutelin fractions were obtain by a triple extraction of rapeseed meal with water (pH 6), 10% NaCl (pH 6), and NaOH (pH 11) respectively. Hydromodules (solid:liquid) 1:10 (10% rapeseed meal), 1:5 and 1:2.5, and extraction continuance of 60 min, 30 min, and 30 min for the first, second, and third extractions were used. The extracts for each fraction were mixed and digestibility was determined as described by Calsamiglia and Stern (1995) with some modifications. Briefly, a sample containing 3% protein was digested with 1.5 mg pepsin (2967.6 U/g, Sigma-Aldrich, St. Louis, MO dissolved in 15 ml 0.1 N HCl) for 3 h at 37ºC. The reaction mixture was neutralized with 0.5 N NaOH followed by an addition of 4 mg pancreatin (903.5 U/g, Sigma-Aldrich, St. Louis, MO) dissolved in 7.5 ml 0.2 M phosphate buffer (pH 8) and 0.005 M sodium azide. After 24 h incubation at 37ºC, non-hydrolyzed protein was precipitated with 10 ml 10% trichloroacetic acid and removed from the reaction mixture by centrifugation for 20 min at 6000 rpm. The degree of hydrolysis was calculated as a ratio of the amount of soluble α-amino nitrogen in the supernatant and the sample N used in the assay. Alfa-amino nitrogen in the supernatant was estimated by ninhydrin method as glycine was used to generate a standard curve (Navarrete del Toro and García-Carreño, 2001). Control experiments without enzyme additions were also performed.

2.5. Statistical analysis

Presented data are averaged means of at least three independent experiments ± standard deviation (SD). Statistical analysis was performed with the program IBM SPSS Stattistics (Somers, NY, USA).

3. Results and discussion

3.1. Production of rapeseed in Bulgaria

Due to its high oil content (42%), rapeseed is an economically important source for production of vegetable oil which is subsequently used for either food or technical purposes. Currently, European Union (27) is a leading producer of biodiesel fuel which is considered an acceptable substitute of paraffinic diesel oil due to similarities in the molecular composition of both fuels (Saka and Kusdiana, 2001). Corn grain ethanol and soybean biodiesel are the two predominant alternative transportation fuels in the United States (Manuel, 2007). In Bulgaria, the production and utilization of biodiesel is stimulated by the National long-term program to promote use of renewable energy sources during 2005-2015 (http://www.strategy.bg/StrategicDocuments/). As a result, the highest production of rapeseed in Bulgaria was observed in 2010 (544 841 t) followed by 2011 (519 910 t) (Fig. 1). After almost a 2-fold decrease during 2012 (271 041 t), the production of the rapeseed during 2014 (498 215 t) almost doubled the one obtained two years ago.

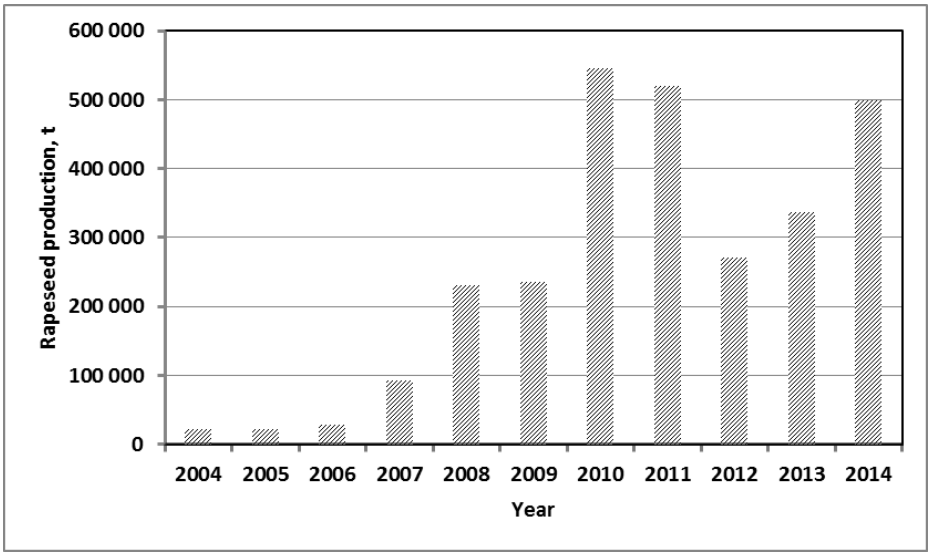

Fig. 1. Rapeseed production in Bulgaria from 2004 to 2014.

Data source: Ministry of Agriculture and Food, Republic of Bulgaria, department of Agrostatistics.

Traditionally, the vegetable oil in Bulgaria is produced mainly from sunflower seeds. The increased interest to rapeseed/canola seeds as an alternative raw material for vegetable fats is determined by higher productivity (kg/ha) and higher demand of rapeseeds at international markets compared to those of the sunflower. However, only a part of the rapeseeds produced in Bulgaria in 2014 was processed with the remaining being exported (Table 1).

Table 1. Rapeseed processing in Bulgaria during 2014.

	Months											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Processed rapeseed, tons	7 093	--	--	--	--	--	5 645	2 555	--	--	2 640	6 000

Data source: National grain department.
Table is incomplete due to data unavailability.

3.2. Biochemical characteristics of rapeseed meal

Biochemical composition of rapeseed meal is important especially when it is used as a source for the production of protein derivatives including isolates, concentrates or hydrolysates for application in food industry. It is characterized with relatively high variability which is partially due to rapeseed varieties used for the production of the rapeseed meal and environmental factors which influence seed development (Bell and Keith, 1991; Li et al., 2015). By studying the nutritive and mineral composition of commercial rapeseed meals from seven canola-crushing plants in Western Canada, Bell and Keith (1991) established high variability in crude protein contents ranging from

37.98 to 43.49%. The commercial rapeseed meal used in our study contained relatively high amount of crude protein (39.86%) which is a necessary prerequisite for a further potential preparation of protein isolates or concentrates for food industry (Table 2).

Table 2. Chemical composition of rapeseed meal

Component	*Content, %
Crude protein	39.86 ± 0.03
Ash	7.07 ± 0.02
Total fats	2.30 ± 0.17
Total fiber	37.69 ± 0.21
Phenols	1.13 ± 0.04
Total glucosinoaltes	< 1 (12.69 ± 0.18 µmol/g)

*Calculated on a dry matter basis (90.46± 0.02%).

According to Ayton (2014), protein content in rapeseeds is genetically determined and is inversely related to oil content. Since the primarily utilization of rapeseeds is the oil production, breeding for varieties with a higher oil content would result to rapeseed meal containing lower protein amount. The high content of total fiber (37.69%, Table 2) is related to the presence of rapeseed hulls in the meal. In contrast to sunflower and soybean seeds, the hulls of the rapeseeds are difficult to remove and therefore, the commercial rapeseed meal is usually undecorticated (Matthäus, 1998). High fiber content of industrially produced rapeseed meal is nutritionally non-advantageous which limits the application of the rapeseed meal in feed industry (Khajali and Slominski, 2012). Although dehulling of rapeseeds was reported to reduce total fiber content by 40% (Kracht et al., 2004), currently it is not practiced under industrial conditions due to possible oil loss and excessive fineness of the rapeseed meal (Khajali and Slominski, 2012). In addition, unit cost of production increases if dehulling is included as a step of the rapeseed meal production (Shires et al., 1983).

It is typical for rapeseed meal that is produced after oil extraction with solvents to contain less than 5% residual oil (Ayton, 2014). The total fats contained in the rapeseed meal evaluated in our study did not exceed 2.30% (Table 2).

Glucosinolates are a large group of sulphur-containing secondary metabolites in cruciferous plants. Once in lower gut, they might be degraded by present microbial population. Resulting break down products, including isothiocyanates, goitrin, nitriles, and thiocyanates could have a negative effect on the function of thyroid gland and overall growth performance (Tripathi and Mishra, 2007). Glucosinolates content is genetically determined and may vary from 7.2 - 30 µmol/g in selected by this parameter varieties (Newkirk et al., 2003) to 120-150 µmol/g in traditional rapeseed varieties (Newkirk, 2009). Industrially produced rapeseed meal used in our study exhibited relatively low total glucosinolate content (12.69 µmol/g), which however, is difficult to relate to a specific variety. Since this specific commercial rapeseed meal is produced of mixed rapeseed cultivars, the lower glucosinolate level is probably due to processing parameters of the oil production (Bell and Keith, 1991). Mansour et al. (1993) reported reduction of glucosinolates contents from 47 to 94% after heat treatment of rapeseeds.

Most of the phenolic compounds available in rapeseeds remain in the meal after oil extraction (Nogala-Kalucka and Siger, 2010). In low concentrations, they act as antioxidants and may prevent food/feed from auto-oxidation. However, in higher amounts they may bind proteins and digestive enzymes thus reducing the nutritional quality of rapeseed protein. In addition, rapeseed phenolics are contributing to the bitter taste, astringency and the dark color of the rapeseed meal thus influencing the overall sensorial perception (Zum Felde et al., 2007). The total phenolics in the rapeseed meal used in our study equaled 1.13%. Therefore, any future potential application as a source for preparation of protein isolates or concentrates for food purposes may require additional steps aiming the reduction of their quantity (Von der Haar et al., 2014).

3.3. Amino acid composition of rapeseed meal

Amino acid analysis of the industrially produced rapeseed meal revealed lysine as a first limiting amino acid with an amino acid score of 58.00% followed by valine (66.86%) (Table 3). The established lysine and valine contents were close to that of the sunflower meal evaluated by Ivanova (2014). The relatively good content of sulphur containing amino acids in the studied rapeseed meal, however, could partially compensate the deficiency of these amino acids observed in the majority of plant proteins (Burstin et al., 2011). The studied rapeseed meal appeared a relatively good source of leucine and isoleucine (Table 3). These amino acids belong to the group of branched chain amino acids. They are primarily metabolized by muscles and stress conditions such as surgery, trauma, infections and starvation require proportionately more leucine and isoleucine than other amino acids (Kinney and Elwyn, 1983). Decreased levels of leucine and isoleucine and therefore increased necessity for external supplementation are observed in patients with liver disease, such as hepatitis, hepatic coma, cirrhosis, and extrahepatic biliary atresia.

Table 3. Essential amino acid composition of rapeseed meal

Amino acid	"Ideal protein", g/100g protein[1]	Rapeseed meal, g/100g protein	Amino acid score, %
Valine	5.0	3.34	66.86
Leucine	7.0	6.83	97.60
Isoleucine	4.0	3.55	88.67
Threonine	4.0	2.84	70.90
Lysine	5.5	3.19	58.00
Methionine + Cysteine	3.5	2.89	82.57
Phenylalanine + Tyrosine	6.0	5.57	92.83

[1]Amino acid composition of an "ideal" protein (FAO, 1970).

In general, arginine and histidine are not considered essential amino acids since they can be synthesized by human body. However, under specific conditions including disease or trauma recovery, the concentration of the arginine in blood plasma may decrease. Because of the absence of any compensatory mechanism, the restoration of the optimum levels of the arginine can be achieved by intake of food rich in this amino acid (Castillo et al., 1994). Like arginine, histidine is a conditionally essential amino acid. While mature individuals can synthesize histidine, infants less than three months old rely on external histidine-rich sources to provide optimum levels of this amino acid required for their metabolism (Pencharz and Ball, 2006). The rapeseed meal protein analysed in this study contained relatively high amounts of arginine (6.01%) and histidine (3.05%) (Table 4) and therefore, could be used as a supplement to improve biological value and amino acid composition of food.

3.4. Digestibility of rapeseed meal protein and rapeseed meal protein fractions

In vitro analysis revealed low digestibility of the commercial rapeseed meal when pepsin and pancreatin were used as proteolytic enzymes (18.59%, Table 5). It is probably due to the conformational changes of proteins that may occur during rapeseeds treatment and oil extraction (Pastuszewska et al., 2003). Interaction of the proteins with other compounds present in the rapeseed meal could also decrease protein digestibility. Rapeseed hulls contain insoluble tannins which amount may reach up to 96% of total tannins (Khajali and Slominski, 2012). They are capable of complexing with proteins including proteolytic enzymes thus decreasing protein digestibility. Low digestibility of rapeseed protein was observed by Bos et al. (2007) who studied the nutritional quality of rapeseed by measuring its real ideal digestibility and postprandial net protein utilization in humans. According to the same authors however, the low digestibility of the rapeseed protein was compensated by excellent postprandial nitrogen retention and high metabolic utilization which was comparable to that of milk protein.

Table 4. Nonessential amino acid composition of rapeseed meal

Amino acid	Rapeseed meal, g/100g protein
Alanine	4.89
Proline	5.85
Glycine	5.38
Serine	3.67
Aspartic acid	12.53
Glutamic acid	12.07
Asparagine	3.84
Arginine	6.01
Histidine	3.05

Table 5. Digestibility of rapeseed meal protein and rapeseed meal protein fractions

Sample	Degree of hydrolysis, %
Rapeseed meal	18.59 ± 0.98
Rapeseed meal albumin	67.22 ± 1.28
Rapeseed meal globulin	63.86 ± 1.86
Rapeseed meal glutelin	47.19 ± 1.95

Higher degrees of hydrolyses were observed at rapeseed protein fractions with albumin and globulin being the most susceptible to pepsin and pancreatin degradation (Table 5). Napin (albumin) and cruciferin (globulin) are the predominant storage proteins of rapeseeds and account for more than 60% of total rapeseed protein (Von der Haar et al., 2014). Similar results were obtained by Nagel et al. (2012) who evaluated the applicability of rapeseed albumin and globulin fractions as a substitute of fish meal in diets fed to rainbow trout. The study demonstrated that albumin concentrate produced from rapeseed meal can be efficiently used to replace 50% of the dietary fish meal in rainbow trout diets. In our study, glutelin fraction exhibited the lowest digestibility (47.19%). This fraction was prepared by alkaline extraction at pH 11 which most probably influenced protein conformation and interaction with other compounds and as a consequence the degree of hydrolysis as well.

4. Conclusions

The commercial rapeseed meal produced in Bulgaria contained a relatively high protein content and low level of anti-nutrient compounds which is an important prerequisite when this by-product is designated for preparation of protein isolates/concentrates for food purposes. Due to the high amounts of leucine, isoleucine and sulphur containing amino acids, the rapeseed meal protein could serve as a supplement to improve biological value and the amino acid composition of food. The relatively high *in vitro* digestibility of albumin and globulin fractions makes the proteins isolated from industrially produced rapeseed meal alternative substitutes for other plant proteins used in food industry.

Acknowledgements

This study was supported by grant № 8/15-H provided by fund “Science”, University of Food Technologies - Plovdiv, Bulgaria.

References

AACC, 1983. Method 46-15: Crude Protein – 5-Minute Biuret Method for Wheat and Other Grains, in *"Approved methods of the American Association of Cereal Chemists"*. American Association of Cereal Chemists, St. Paul, MN, USA.

Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of Total Phenolic Content and Other Oxidation Substrates in Plant Tissues Using Folin–Ciocalteu Reagent. Nature Protocols 2, 875-877.

Ayton, J., 2014. Variability of Quality Traits in Canola Seed, Oil and Meal - A Review. NSW Department of Primary Industries, New South Wales, Australia.

Bell, J.M., Keith, M.O., 1991. A Survey of Variation in the Chemical Composition of Commercial Canola Meal Produced in Western Canadian Crushing Plants. Canadian Journal of Animal Science 71, 469-480.

Bell, J.M., Jeffers, H.F., 1976.Variability in the Chemical Composition of Rapeseed Meal. Canadian Journal of Animal Science 56, 269-273.

Bellostas Muguerza, N., Sørensen, H., Sørensen, S., 2007. Quality of Rapeseed Oil for Non-Food (Bioenergy), and Human and Animal Nutrition, Nutrition and Processing Workshop of the International Rapeseed Congress, Wuhan, China.

Bos, C., Airinei, G., Mariotti, F., Benamouzig, R., Bérot, S., Evrard, J., Fénart, E., Tomé, D., Gaudichon, C., 2007. The Poor Digestibility of Rapeseed Protein is Balanced by Its Very High Metabolic Utilization in Humans. The Journal of Nutrition 137, 594-600.

Burstin, J., Gallardo, K., Mir, R.R., Varshney, R.K., Duc, G., 2011. Improving Protein Content and Nutrition Quality, in *"Biology and Breeding of Food Legumes"*, In: Pratap, A., Kumar, J. (Eds.), CAB International, Wallingford, UK, 314-328.

Calsamiglia, S., Stern, M.D., 1995. A Three-Step In Vitro Procedure for Estimating Intestinal Digestion of Protein in Ruminants. Journal of Animal Science 73, 1459-1465.

Carré ,P., Pouzet, A., 2014. Rapeseed Market, Worldwide and in Europe. OCL-Oilseeds and Fats, Crop and Lipids 21, D102.

Castillo, L., Ajami, A., Branch, S., Chapman, T.E., Yu, Y., Burke, J.F., Young, V.R., 1994. Plasma Arginine Kinetics in Adult Man: Response to an Arginine-Free Diet. Metabolism 43, 114-122.

FAO, 1970. Nutritional Studies №24 Amino Acid Content of Foods and Biological Data on Proteins. FAO, Rome.

ICC Standard №104/1, Approved 1960, Revised 1990. Determination of Ash in Cereals and Cereal Products.

ICC Standard №156, Approved 1994. Determination of Total Dietary Fiber.

ISO 7302:2003 Cereals and Cereal Products -Determination of Total Fat Content.

Ivanova, P., 2014. Preparation, Characterization and Enzymatic Modification of Protein Isolates from Sunflower Meal. Dissertation, University of Food Technologies, Plovdiv, Bulgaria.

Ivanova, R., 2012. Рапицата – култура на настоящето и бъдещето. "Виденов и син", София, ISBN: 978-954-8319-59-1306 (BG).

Jezek, J., Haggett, B.G.D., Atkinson, A., Rawson, D.M., 1999. Determination of Glucosinolates Using Their Alkaline Degradation and Reaction with Ferricyanide. Journal of Agricultural and Food Chemistry 47, 4669-4674.

Khajali, F., Slominski, B.A., 2012. Factors That Affect the Nutritive Value of Canola Meal for Poultry. Poultry Science 91, 2564-2575.

Kinney, J.M., Elwyn, D.H., 1983. Protein Metabolism and Injury. Annual Review of Nutrition 3, 433-466.

Kracht, W., Dänicke, S., Kluge, H., Keller, K., Matzke, W., Hennig, U., Schumann, W., 2004. Effect of Dehulling of Rapeseed on Feed Value and Nutrient Digestibility of Rape Products in Pigs. Archives of Animal Nutrition 58, 389-404.

Li, P., Wang, F., Wu, F., Wang, J., Liu, L., Lai, C., 2015. Chemical Composition, Energy and Amino Acid Digestibility in Double-Low Rapeseed Meal Fed to Growing Pigs. Journal of Animal Science and Biotechnology 6, 37-46.

Mansour, E.H., Dworschák, E., Lugasi, A., Gaál, Ö., Barna, É., Gergely, A.,1993. Effect of Processing on the Antinutritive Factors and Nutritive Value of Rapeseed Products. Food Chemistry 47, 247-252.

Manuel, J., 2007. Battle of the Biofuels. Environmental Health Perspectives 115, A92-A95.

Matthäus, B., 1998. Effect of Dehulling on the Composition of Antinutritive Compounds in Various Cultivars of Rapeseed. Lipid/Fett 100, 295 - 301.

Ministry of Agriculture and Food, Republic of Bulgaria, 2015. Agricultural Report 2015, http://www.mzh.government.bg/mzh/bg/Documents/AgrarenDoklad.aspx, Accessed: December 2015.

Nagel, F., Slawski, H., Adem, H., Tressel, R., Wysujack, K., Schulz, C., 2012. Albumin and Globulin Rapeseed Protein Fractions as Fish Meal Alternative in Diets Fed to Rainbow Trout (*Oncorhynchus mykiss* W.). Aquaculture 354–355, 121-127.

Navarrete del Toro, M.A., García-Carreño, F.L., 2001. Evaluation of the progress of protein hydrolysis.Current, in *"Protocols in Food Analytical Chemistry"*. Supplement 4. John Wiley & Sons, Inc., B2.2.1-B2.2.14.

Newkirk, R., 2009. Canola Meal: Feed Industry Guide, Canadian International Grains Institute, www.canolacouncil.org

Newkirk, R.W., Classen, H.L., Scott, T.A., Edney, M.J., 2003. The Digestibility and Content of Amino Acids in Toasted and Non-Toasted Canola Meals. Canadian Journal of Animal Science 83, 131-139.

Newkirk, R.W., Classen, H.L., 2002. The Effects of Toasting Canola Meal on Body Weight, Feed Conversion Efficiency, and Mortality in Broiler Chickens. Poultry Science 81, 815-825.

Nogala-Kalucka, M., Siger, A., 2010. Changes of Phenolic Content in Rapeseed During Preliminary Drying. Journal of Oilseed Brassica 1, 33-38.

Pastuszewska, B., Jabłecki, G., Buraczewska, L., Dakowski, P., Taciak, M., Matyjek, R., Ochtabińska, A., 2003. The Protein Value of Differently Processed Rapeseed Solvent Meal and Cake Assessed by In Vitro Methods and in Tests with Rats. Animal Feed Science and Technology 106, 175-188.

Pencharz, P.B., Ball, R.O., 2006. Amino Acid Requirements of Infants and Children, in *"Protein and Energy Requirements in Infancy and Childhood"*, In: Rigo, J., Ziegler, E.E. (Eds.), Nestlé Nutrition Workshop Series Pediatric Program. Nestec Ltd., Vevey/S.Karger AG, Basel, pp. 109-119.

Saka, S., Kusdiana, D., 2001. Biodiesel Fuel From Rapeseed Oil as Prepared in Supercritical Methanol. Fuel 80, 225-231.

Shires, A., Bell, J.M., Laverty, W.H., Fedec, P., Blake, J.A., McGregor, D.I., 1983. Effect of Desolventization Conditions and Removal of Fibrous Material by Screening on the Nutritional Value of Canola Rapeseed Meal for Broiler Chickens. Poultry Science 62, 2234-2244.

Tan, S.H., Mailer, R.J., Blanchard, C.L., Agboola, S.O., 2011. Canola Proteins for Human Consumption: Extraction, Profile, and Functional Properties. Journal of Food Science 76, R16-R28.

Tomov, T., Rachovski, G., Kostadinova, S., Manolov, I., 2009. Handbook of Agrochemistry. Academic publisher of Agricultural University Plovdiv., Plovdiv, Bulgaria, p. 109.

Tripathi, M.K., Mishra, A.S., 2007. Glucosinolates in Animal Nutrition: A Review. Animal Feed Science and Technology 132, 1-27.

Villanueva, A., Clemente, A., Bautista, J., Millán, F., 1999. Production of an Extensive Sunflower Protein Hydrolysate by Sequential Hydrolysis with Endo- and Exo-Proteases. Grasas y Aceites 50, 472-476.

Von der Haar, D., Müller, K., Bader-Mittermaier, S., Eisner, P., 2014. Rapeseed proteins – Production Methods and Possible Application Ranges. OCL - Oilseeds and Fats, Crops and Lipids, 21, D104.

Zum Felde, T., Baumert, A., Strack, D., Becker, H.C., Möllers, C., 2007. Genetic Variation for Sinapate Ester Content in Winter Rapeseed (*Brassica Napus* L.) and Development of NIRS Calibration Equations. Plant Breeding 126, 291-296.