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REVIEW



Canola/rapeseed protein – nutritional value, functionality and food application: a review

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ABSTRACT

Plant-based diet and plant proteins specifically are predestined to meet nutritional requirements of growing population of humans and simultaneously reduce negative effects of food production on the environment. While searching for new sources of proteins, special emphasis should be placed on oilseeds of Brassica family comprising varieties of rapeseed and canola as they contain nutritionally valuable proteins, which have potential to be used in food, but are now rarely or not used as food components. The purpose of the present work is to provide a comprehensive review of main canola/rapeseed proteins: cruciferin and napin, with the focus on their nutritional and functional features, putting special emphasis on their possible applications in food. Technological challenges to obtain rapeseed protein products that are free from anti-nutritional factors are also addressed. As molecular structure of cruciferin and napin differs, they exhibit distinct features, such as solubility, emulsifying, foaming or gelling properties. Potential allergenic effect of 2S napin has to be taken under consideration. Overall, rapeseed proteins demonstrate beneficial nutritional value and functional properties and are deemed to play important roles both in food, as well as, non-food and non-feed applications.

KEYWORDS

Plant protein; protein isolates; napin; cruciferin; rapeseed

Introduction

One of the most challenging issues of the world today is to provide an access to nutritious, sustainable and sufficient foods to growing Earth population. (FAIRR 2018). The Food and Agriculture Organization (FAO) of the United Nations has stated that population requirements for animal proteins are predicted to double by 2050 (Boland 2013). Food production industry is responding to the demand challenge (FAIRR 2018). The global production of meat is predicted to go up by 13% in 2026, in comparison to 2014–2016. This is in agreement with a nearly 20% increase over the past ten years (OECD-FAO 2017). Alongside the growing population, the impact on the environment of food production is also on the rise. The meat production has negative influence on groundwater, soil or tropical forests and leads to nitrogen, carbon and phosphorus cycles disruption (Aiking 2011; Smil 2014). Moreover, livestock greenhouse gas emissions are similar to these from global transportation and constitute 14% of worldwide emissions (Tirado et al. 2018). It has become obvious that food production needs to be more sustainable and one of the hopeful idea in human nutrition is to partially replace animal derived proteins with these of plant origin. It is claimed that this change would help to conserve broadly defined environmental welfare (Aiking 2011). The increase of plant-based protein supply in diet is

not only environmentally responsible but also possesses beneficial impact on human's health. According to Marsh, Munn, and Baines (2013) this approach may give a support in weight management and reduce the risk of some chronic diseases that are related to eating habits. In this respect, the plant protein nutritional value is of utmost importance. Consumption of proteins from alternative sources and flexitarian diets have been recognized as crucial food trends in the coming years and the rapid growth on the plant-derived food market is already observed (Campbell, Rempel, and Wanasundara 2016; FAIRR 2018).

Rapeseed, being the world's second produced oilseed, is the promising source of a novel protein, due to its great nutritional value, expressed in well-balanced amino acid profile and highly desirable technological and functional properties. Rapeseed can be also an alternative in the plant-based market dominated by soy protein. Besides rapeseed's high production volumes, it is also well adapted to different agricultural conditions (Carré and Pouzet 2014; Von Der Haar et al. 2014; Rodrigues et al. 2012; Mupondwa, Li, and Wanasundara 2018). Rapeseed is a herbaceous, annual plant, which belongs to *Brassicaceae* family. It is primarily cultivated for seeds containing approximately 40–50% of edible oil (Lim 2013). Canola (Canadian oil, low acid) is the derivative of rapeseed, that was obtained by classical

breeding methods, with the aim of improving quality of oil by reduction of the content of anti-nutritional compounds: glucosinolates and erucic acid. The level of glucosinolates accounts typically for $<30 \mu\text{mol/g}$ in the meal residue and the content of erucic acid is below 2% in the oil. The term 'canola' refers to *Brassica napus* and *Brassica rapa* refined germ plasm. The term 'canola-quality juncea' was also developed to describe *Brassica juncea* improved germ plasm. However, it is *Brassica napus* canola, which is primarily cultivated and thus investigated worldwide. For example, over the 95% seeds produced in Canada are *B. napus*. Canola is mostly used term in Canada and Australia, whereas in Europe the name rapeseed or 'double-zero rapeseed' (low content of both glucosinolates and erucic acid) is preferred (Rodrigues et al. 2012; CCC 2015; Wanasundara et al. 2016). Hereafter, both terms are used interchangeably.

The left-overs after pressing oil from rapeseed, either in a form of de-oiled meal or a cake with higher residual oil content after mechanical pressing, are nowadays used mostly as animal feed in ruminant, swine, poultry or aquaculture diets. So far, there are no reports on the use of this material as a source of food on a commercial scale, even though it contains nutritionally complete proteins adequate to meet human demand for essential amino acids, as was demonstrated in previous studies (Wanasundara et al. 2016; CCC 2015; Fleddermann et al. 2013). However, there are several companies, which are interesting in bringing rapeseed proteins to the market and the technology of obtaining rapeseed protein is becoming more and more advanced (Mupondwa, Li, and Wanasundara 2018). There is some new market information indicating that it is possible to retrieve all protein contained in rapeseed in the form of food-grade protein products by using novel technology (www.napiferyn.pl). This all indicates that rapeseed protein has opportunity to be successfully implemented as food ingredients (Mupondwa, Li, and Wanasundara 2018). The present work provides a review of main canola/rapeseed seed storage proteins: cruciferin and napin, with regard to their nutritional value and functional properties, putting special emphasis on possible uses in food applications.

Canola/rapeseed protein

Rapeseed contains 17–26% of protein (Li et al. 2012). In Canada, over the period 2000–2015, proteins constituted 37.0–41.4% of produced meal at 12% moisture (Barthet 2015). The majority, approximately 90%, of proteins found in rapeseed are of storage nature (Wanasundara et al. 2017). They are deposited into the matrix and crystalloid structures located in protein storage vacuoles (Jiang et al. 2001). In rapeseed, the predominant storage proteins are cruciferin and napin. Their molecular weight are reported to be 300–350 kDa and 12–16 kDa (Wanasundara et al. 2016); approximately 300 kDa and 14 kDa (GRAS 2016); about 300 kDa and 12–14 kDa (Von Der Haar et al. 2014). In European varieties they appear in the ratio varied from 0.6 to 2. In *Brassica napus*, apart from storage proteins, structural oil body proteins and metabolic (for example lipid transfer) proteins are also present and may

be recovered from seed or meal during technological process (Wanasundara et al. 2016; Wanasundara 2011).

Cruciferin

According to Perera, McIntosh, and Wanasundara (2016) cruciferin (11S globulin) belongs to cupin superfamily. Different levels of cruciferin structure are well described (Wanasundara 2011). According to Wanasundara et al. (2017), 9–12 genes are involved in encoding cruciferin subunits. This protein is composed of 6 protomers (or subunits), which are built by 2 trimers and each protomer contains 2 polypeptides. Polypeptide α (approximately 40 kDa) is comprised of 254–296 amino acids, whereas polypeptide β (approximately 20 kDa) is composed of 189–191 amino acids. These chains are linked together by disulfide bonds and 2 trimers are primarily stabilized by non-covalent bonds. Reported cruciferin subunits have been named CRUA, CRU1, CRU2, CRU3 and CRU4 (Wanasundara et al. 2016). The cruciferin secondary structure is comprised of 27 β strands and 7 helices, which are folded into domains: 2 β -barrel and 2 extended helices. The mature subunit of cruciferin possesses molecular mass ranging from 48 to 56 kDa and has its isoelectric point at $\text{pH} = 7.25$ (Wanasundara et al. 2017). According to Wanasundara (2011), the temperature which causes denaturation of 11S globulin is equal to 80°C for $\text{pH} = 6$ and 81.3°C for $\text{pH} = 10$. The crystal structure of *Brassica napus* procruciferin (11S globulin) is presented in the Figure 1.

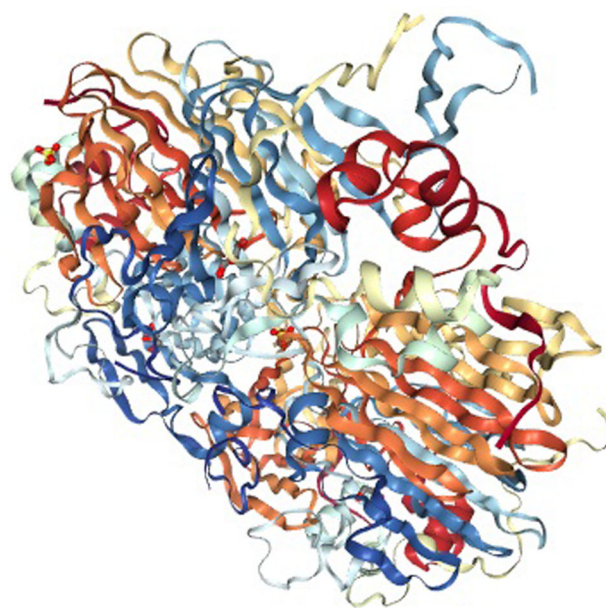


Figure 1. The crystal structure of *Brassica napus* procruciferin. Source: Image of PDB entry 3KGL (Tandang-Silvas, M. R. G., T. Fukuda, C. Fukuda, K. Prak, C. Cabanos, A. Kimura, T. Itoh, B. Mikami, S. Utsumi, N. Maruyama. 2010. Conservation and divergence on plant seed 11S globulins based on crystal structures. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1804: 1432–42) was created using the NGL Viewer (Rose A. S, A.R. Bradley, Y. Valasatava, J. M. Duarte, A. Pilić, and P. W. Rose. 2018. NGL viewer: web-based molecular graphics for large complexes. *Bioinformatics*. doi:10.1093/bioinformatics/bty419) at the RCSB PDB (www.rcsb.org).



Figure 2. The structure of napin BnIb (NMR). *Source:* Image of PDB entry 1PNB (Rico, M., M. Bruix, C. González, R. I. Monsalve, and R. Rodríguez. 1996. 1H NMR assignment and global fold of napin BnIb, a representative 2S albumin seed protein. *Biochemistry* 35: 15672-82) was created using the NGL Viewer (Rose A. S, A.R. Bradley, Y. Valasatava, J. M. Duarte, A. Prlić, and P. W. Rose. 2018. NGL viewer: web-based molecular graphics for large complexes. *Bioinformatics*. doi:10.1093/bioinformatics/bty419) at the RCSB PDB (www.rcsb.org).

Napin

According to Wanasundara et al. (2017) napin (2S albumin) is classified under the prolamin superfamily. This protein is encoded by 10–16 genes (Wanasundara 2011). The structure of mature napin consists of 2 polypeptide chains – one short (4 kDa) and a long one (9 kDa) which are linked together by two disulfide bonds (inter-chain). Long chain contains also 2 disulfide bonds (intra-chain), which bind residues of cysteine. They stabilize the molecule by developing 4 disulfide bridges (Wanasundara et al. 2016). According to Perera, McIntosh, and Wanasundara (2016) in *Brassica napus* a few isoforms have been identified as follows: Napin-1, Napin-2, Napin-3, Nap1, Napin-1A and Napin-B. The secondary structure of napin has been reported as 38% β -sheet and 25% α -helix composition (Wanasundara 2011). According to Von Der Haar et al. (2014) napin possesses isoelectric point above pH = 10 and remains stable up to 75 °C (Wanasundara et al. 2017). The structure of napin BnIb (NMR) is presented in the Figure 2.

Oil body proteins

Oil bodies (lipid bodies, oleosomes) are intra-cellular organelles which stored neutral lipids (Tzen 2012). Oleosomes can be retrieved by aqueous extraction, together with other seed constituents, for example protein (Romero-Guzmán et al. 2020). They are primarily consisted of triacylglycerols and sterol esters, surrounded by phospholipids, in which integral protein are included. These proteins provide oil bodies stability (Pasaribu et al. 2017). Oil body proteins are comprised of hydrophobic and hydrophilic domains. Long hydrophobic domain can bond with droplet's lipid phase and hydrophilic domain is presented on the surface of oil bodies. In *Brassica napus*, the following oil body proteins have been identified: oleosins, caleosins and steroleosins (Wanasundara et al. 2016). According to Tzen (2012) several other proteins, which can potentially be reported as oils body proteins, have been also found in rapeseed. Oleosins, which possess low

molecular mass, approximately 18–25 kDa, are predominant (up to 90%) rapeseed oil body proteins (Jolivet et al. 2009). Due to their specific structure, resembling surfactant with hydrophilic head and hydrophobic chain, they possess high interfacial activity and emulsifying properties, which can be used in different food emulsions (Nikiforidis 2019; Östbring, Tullberg, Burri, et al. 2019) Caleosins are characterized with molecular mass of approximately 30 kDa and possess the ability to bind calcium. Steroleosins with molecular mass of approximately 40 kDa have been much less investigated as compared to oleosins and caleosins (Pasaribu et al. 2016; Tzen 2012).

Protein isolates and concentrates from canola/rapeseed

According to Belitz, Grosch, and Schieberle (2009) protein concentrates are obtained by extraction of mainly non-protein components from source material, whereas protein isolates are received by extraction and subsequent isolation of protein from the solution using adequate techniques such as precipitation or thermal coagulation. According to Akbari and Wu (2015) protein extraction from rapeseed is perceived to be a challenge. It is mostly because of broad range of proteins exhibiting different properties but also due to occurrence of different anti-nutritional compounds, including carbohydrates, phytates, phenolic compounds and glucosinolates. It has been reported that ultrafiltration and diafiltration can significantly reduce the level of some of these anti-nutritional factors (Gerzhova et al. 2015a).

Various methods of obtaining protein-rich products from rapeseed have been reported. The most common approach is direct extraction at alkaline pH and subsequent isoelectric precipitation at acidic conditions. Since after the precipitation, proteins of prospectively high functional value remain in the solution, the possibility of obtainment of two protein-rich product simultaneously and thus more complete utilization of source material has been pointed out (Ivanova et al. 2017; Gerzhova et al. 2015a). Nevertheless, Gerzhova et al. (2015a) have indicated that despite of high level of extractability (up to 60–80%), eminently alkaline conditions may lead to protein denaturation, decrease in essential amino acids level and lower digestibility. These conditions may also contribute to dark color and negative flavor, due to better extractability of phenolic compounds at basic pH (Xu and Diosady 2000). What is more, using standard protocol might be insufficient to reduce the level of anti-nutritional or even toxic compounds, such as glucosinolates or their degradation products. Despite high nitrogen yield, the alkaline extraction protocol is deemed inappropriate to produce protein products derived from rapeseed aimed for use in food. In order to improve the quality, different reagents and various conditions have been tested, such as addition of salts, variation of extraction time and temperature, or testing different meal-to-solvent ratios. Alternative processing techniques include, for example membrane separation, protein micellation and electro-activation technologies, or even usage of enzymes (Gerzhova et al. 2015a; Tan et al. 2011a). One essential problem related to processing of hexane-extracted canola

meals is the fact that proteins may undergo temperature-induced damage in a desolventizer/toaster step that is routinely employed to remove hexane solvent from the meal. Several studies reported that the use of solvents and heat treatment decrease emulsifying abilities of rapeseed protein (Östbring, Tullberg, Burri, et al. 2019). On the other hand, the content of oil in cakes obtained by mechanical expeller pressing might be too high and therefore another step in the process might be required to remove it. One promising alternative could be cold pressing and desolventizing at low temperature (Campbell, Rempel, and Wanasundara 2016). The recent study performed by Östbring, Malmqvist, Nilsson, et al. (2019) indicated that cold-pressed rapeseed press cake may be the most suitable starting material for obtaining high quality rapeseed protein in relation to emulsifying abilities, particularly if the recovery process is also performed at moderated temperatures. They demonstrated that the highest protein recovery was observed (45%) for the protocol without heat exposure. That to be compared with hot-pressed rapeseed meal and solvent-extracted rapeseed meal that yielded protein recovery equal to 26% and 5%, respectively. When the heating was applied in the protein recovery process, a slight increase in the protein yield was found for solvent-extracted rapeseed meal, while a decrease in yield was observed for cold-pressed cake. For hot-pressed meal, no significant changes due to the use of heat in the extraction process were detected.

Recently, Zhang, He, et al. (2020) who examined different properties of protein recovered by alkaline extraction (pH 8.0–13.0) and subsequent acidic precipitation (pH 3.0–5.5), stated that by means of this method, safe, bioavailable and intact protein can be obtained. They stated that pH adjustment can be a suitable method to retrieve proteins with high functional properties. They pointed out that extraction at pH = 9 and subsequent precipitation at pH = 4.5 had the highest yield and exhibited low content of antinutritional compounds.

Until now, several commercial protein products derived from canola have been described. Among these, products under commercial names: SuperteinTM, Puratein[®] (Burcon, Nutrascience), IsolexxTM and VitalexxTM – hydrolyzed isolate (BioExx), and CanolaPROTM (DSM) (Wanasundara et al. 2016; GRAS 2011; GRAS 2016). SuperteinTM is consisted of > 80% of napin fraction, whereas Puratein[®] consists > 80% of cruciferin fraction. IsolexxTM is mainly composed of 60–65% of globulin and 30–35% of albumins. CanolaPROTM contains approximately 35–60% napins and 40–65% cruciferins. In all mentioned products, proteins constitute min. 90%. (EFSA 2013; Wanasundara et al. 2016; GRAS 2016). Another company involved in development of technologies for rapeseed protein production is NapiFeryn BioTech. As described by Ostrowska, Kozłowska, Rachwał-Rosiak, et al. (2018), an isolate obtained by the company's technology possesses high content of protein and low content of anti-nutritional compounds. The company also introduced novel rapeseed protein-fiber concentrate aimed for use in food (Ostrowska, Kozłowska, Rachwał, et al. 2018).

The European Commission, on 1 July 2014, adopted a decision, addressed to Siebte PMI Verwaltungs GmbH, Neuer

Table 1. Specification of rapeseed protein.

Description: White to off-white, spray dried powder	
Total protein	≥90%
Soluble protein	≥85%
Moisture	≤7%
Carbohydrates	≤7%
Fat	≤2%
Ash	≤4%
Fiber	≤0.5%
Total glucosinolates	≤1 mmol/l
Total phytate	≤1.5%
Lead	≤0.5 mg/kg
Yeast and mold count	≤100 cfu/g
Aerobic bacteria count	≤10,000 cfu/g
Total coliform count	≤10 cfu/g
<i>Escherichia coli</i>	Absent in 10 g
<i>Salmonella</i> spp.	Absent in 25 g

Source: European Commission (2014).

Jungfernstieg 5, 20354 Hamburg, Germany, “authorising the placing on the market of rapeseed protein as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council” Rapeseed protein is defined as ‘an aqueous protein-rich extract from rapeseed press cake originating from non-genetically modified *Brassica napus* L. and *Brassica rapa* L.’ To be placed on the market, product must be consistent with the specification, as given in Table 1 (European Commission 2014).

Nutritional value

Amino acid composition

Amino acids are protein building subunits, therefore their composition has crucial impact on protein quality. Due to well-balanced amino acid profile, it has been reported that rapeseed proteins exhibit great nutritional value and can satisfy human needs for essential and non-essential amino acids. The content of essential amino acids in rapeseed products are over 400 mg/g protein, including cysteine and tyrosine. They are also characterized by high level (40–49 mg/g protein; 3.0–4.0%) of sulfur containing amino acids – methionine and cysteine, which exceeds the requirements established by WHO/FAO/UNU and makes them superior to other available plant proteins, including legumes. Nevertheless, lysine has been determined as first limiting amino acid and its temperature sensitivity and possibility to undergo some chemical reactions, for example Maillard reaction, have been reported (Tan et al. 2011a; Wanasundara et al. 2016; Fleddermann et al. 2013; Alashi et al. 2013). The content of particular amino acids in commercial rapeseed products is shown in Table 2. As can be noticed, the level of amino acid is varied for different types of protein (albumin:globulin ratio), however in all products the highest content of glutamic acid/glutamine (over 19 g/100 g protein) is observed. Napin-rich protein isolate SuperteinTM possesses the highest amount of lysine and sulfur – containing amino acids, whereas the highest level of aromatic amino acids (phenylalanine, tryptophan and tyrosine) is estimated for cruciferin-rich protein isolate Puratein[®]. All of presented rapeseed products meet the requirements for adults with regard to indispensable amino acids, except for Puratein[®] for lysine.

Table 2. Comparison of the amino acid composition of various protein isolates. Values are reported as g/100g protein (or product).

	Indispensable amino acid requirements for adults ^{a,***}	Supertein ^{TM,b}	Puratein ^{®b}	Isolexx ^{TM,c}	Canola PRO ^{TM,d}
Alanine	–	4.0	4.2	4.5	4.17
Arginine	–	5.8	7.2	7.6	6.32
Aspartic acid/Asparagine	–	2.6	9.3	8.8	5.68
Cystine/cysteine	0.6	4.5	1.6	2.0	3.51
Glutamic acid/Glutamine	–	24.6	19.8	19.8	22.4
Glycine	–	4.3	5.4	5.4	4.83
Histidine*	1.5	3.6	2.5	3.1	3.05
Isoleucine*	3.0	3.0	4.4	4.2	3.52
Leucine*	5.9	6.0	8.2	7.8	6.84
Lysine*	4.5	7.4	4.0	5.5	6.08
Methionine*	1.6	2.4	1.9	2.0	2.06
Phenylalanine*	3.8**	2.6	4.9	4.4	3.65
Proline	–	9.2	5.8	5.8	6.57
Serine	–	3.3	4.1	4.9	3.91
Threonine*	2.3	3.2	3.7	4.5	3.73
Tryptophan*	0.6	1.4	2.0	1.5	1.36
Tyrosine	3.8**	1.4	4.1	3.3	1.96
Valine*	3.9	4.3	5.5	5.0	4.69

^aWHO/FAO/UNU (2007).^bGRAS (2010).^cGRAS (2011).^dGRAS (2017).

*Essential amino acid.

**Phenylalanine + tyrosine.

***Mean daily nitrogen demand – 105 mg/kg (0.66 g protein/kg).

Digestibility

Digestibility of proteins can be measured by different rates, including PDCAAS (protein digestibility corrected amino acid score) and DIAAS (digestible indispensable amino acid score). PDCAAS score is estimated by dividing the level of the first limiting dietary essential amino acid by requirement of amino acid for child in age of 1–2 and correcting for protein digestibility. DIAAS score takes into account true ileal amino acid digestibility calculated for particular amino acid and accessibility of lysine. Prevailing FAO endorsement is to use DIAAS, which utilizes ileal digestibility, rather than PDCAAS based on fecal digestibility, mainly because ileal digestibility is more accurate than fecal digestibility and amino acid digestibility is more precise than nitrogen-based protein digestibility (Wanasundara et al. 2016; Rutherford et al. 2015). Rutherford et al. (2015) claimed that levels of true ileal amino acid digestibility determined in various products of plant origin (soy protein isolate, rice and pea protein concentrates, cooked kidney beans, peas, rolled oats and rice, roasted peanuts, wheat bran and corn cereals) and animal – derived (whey protein isolate and concentrate, milk protein concentrate) reported for rat models were comparable to previously presented data for adult human. Taking this into account, it has been stated that animal models can be used for accurate calculations of canola proteins nutritional value (Wanasundara et al. 2016).

Availability and activity of enzymes plays crucial role in protein digestibility, which is connected with chemical composition and anti-nutritional factors. High content of fiber and presence of glucosinolates have been reported to be a reason for lower digestibility of rapeseed meal as compared to soybean meal. While feeding test's animals with rapeseed meal, glucosinolates and their degradation products such as isothiocyanates have been claimed to cause such negative

effects as growth reduction and enlargement of thyroid. These constituents are also claimed responsible for decreasing the activity of proteolytic enzymes and binding the proteins, which prevents enzyme-based hydrolysis. Nutritional value and digestibility may also be reduced due to lengthy process especially when high temperature and alkaline conditions are being utilized, as they may yield adverse chemical reactions such as amino acid racemization and formation of lysinoalanine. Moreover, lower content of digestible amino acids has been observed in conventionally toasted canola meal as compared to non-toasted one. Mean value for digestibility for toasted meal has been equaled 78.0%, whereas non-toasted meal – 89.7%. It is with agreement with other conducted studies which showed that toasting used in rapeseed meal production may diminish ileal digestibility of amino acid and crude protein, however Salazar-Villanea et al. (2016) claimed that although increasing toasting time leads to protein changes, which have impact on nutritional value, these modifications are correlated with hydrolysis rate, but not the range of this process, or in vitro digestibility of crude protein. Also in vitro digestibility of canola meal subjected to different extrusion conditions has been studied. The research revealed digestibility improvement after treatment at 30% barrel moisture and noticed complex reliance between process conditions and constituents interactions. Considering all these results, obtaining of highly purified products by suitable and gentle treatment, for example isolates with high content of protein and low content of anti-nutritional compounds appears to be of utmost challenge (Wanasundara et al. 2016; Achary and Thiyam 2012; Rodrigues et al. 2012; Newkirk et al. 2003; Salazar-Villanea et al. 2016; Zhang et al. 2017).

Several studies on digestibility of canola protein products were carried out in 1980s and 1990s. Lower in vitro

digestibility was reported for canola protein concentrate (52% of protein content) than for casein, 83% and 97% respectively, which was supposedly caused by characteristic structural properties of canola proteins which result in endurance to acidic denaturation and unfolding. Canola proteins also presented poor *in vivo* digestibility in rats and pigs and the resistant to hydrolysis portions of protein were observed in digested products. Whilst estimating PER (protein efficiency ratio) of rapeseed 12S, 2S protein and rapeseed flour along with casein under the same conditions, following values were determined: 2.12, 2.49, 2.64 and 3.23, respectively. Low digestibility of canola proteins was also found in cockerels and chicks (Wanasundara et al. 2016). However, Bos et al. (2007) highlighted that lower digestibility may be balanced by great metabolic usage in human. According to Mariscal-Landín et al. (2008) canola meal exhibits poorer digestible energy content as compared to soybean meal, due to high level of NDF (neutral detergent fiber) provided by significant amount of hulls constituting approximately 30% of rapeseed meal. This research group presented that canola meal proteins digestibility was lower in weaned piglets in comparison to growing pigs, however this factor might be improved for weaned piglets by pelleting the meal, which can diminish loss of endogenous proteins by altering the matrix of fiber. This effect might also be noticed for dehulled meal. The studies on crude protein digestibility in ruminants were also conducted. Ebrahimi et al. (2009) stated that γ -irradiation of canola seeds increased intestinal digestibility of crude proteins. What is more, a possible reduction of glucosinolates and phytic acid in canola seeds was suggested. In contrast to previously mentioned studies, Boutry et al. (2011) in their research in rats, presented that both rapeseed protein isolate and milk protein isolate possessed similar true protein digestibility of high value equaled approximately 95%. They revealed comparable levels of *in vivo* postprandial dietary nitrogen retention and digestion in examined proteins, but highlighted that rapeseed protein isolate retain nitrogen especially in splanchnic organs, such as liver or intestinal mucosa, whereas milk protein isolate – more in the skin. Fleddermann et al. (2013) determined true digestibility of canola protein in rats and received the value of 93.3% for isolate and 97.3% for hydrolysate.

Digestibility of rapeseed products in pigs was also investigated by Kasprzak et al. (2018). They studied standardized ileal digestibility and the content of amino acids and crude protein in different rapeseed co-products. The research showed that digestibility can be enhanced when cooking step and heat treatment of seed is eliminated. It was also claimed that neutral detergent fiber (NDF) content had negative impact on digestibility, so it can be good indicator of crude protein and amino acids digestibility.

Some data on commercial rapeseed protein products are also available. PDCAAS values for SuperteinTM and Puratein[®], calculated according to WHO/FAO guidelines from 1989 are 61% and 64%, respectively. The most limiting amino acids in SuperteinTM are phenylalanine and tyrosine, but also leucine and threonine, whereas in Puratein[®] it is found to be lysine. When calculating PDCAAS of these proteins according to

updated standards from 2002, estimated values are respectively for SuperteinTM and Puratein[®]: 83% and 71% for children at the age of 1–2, yet 92% and 77% for 3–10 years old (GRAS 2010). In a 13-week dietary toxicity assessment in rats, SuperteinTM did not exhibit adverse effects at administration dose of 20% w/w and no effects were observed at 5% w/w feeding level. In a dietary toxicity sub-chronic research by 13-week rats feeding, the “no-observed-effect” and “no-observed-adverse-effect” amount for Puratein[®] were estimated to be 10% w/w, and 20% w/w respectively. With regard to these studies both isolates were considered as safe (Mejia, Korgaonkar, Schweizer, Chengelis, Novilla, et al. 2009; Mejia, Korgaonkar, Schweizer, Chengelis, Marti, et al. 2009). Estimated PDCAAS value of IsolexxTM is 1.04, with lysine as limiting amino acid and true digestibility over 90% (GRAS 2011). The digestibility of CanolaPROTM is 87%, PDCAAS value for 2–5 years old equals 87%, and DIAAS for children at the age of 0.5–3.0 is estimated to be 95%, which is similar to soybean isolates. According to FAO regulations, DIAAS value ranging from 75% to 99% points out a good protein source (GRAS 2016).

Bos et al. (2007) reported, for the first time, evaluation of rapeseed protein nutritional value in human. Several rates, such as real ileal digestibility (RID), net postprandial protein utilization (NPPU) or postprandial biological value (PBV) were determined. Rapeseed possessed the lowest RID (84%) of all presented protein sources, which were milk (95%), soy (91.5%), wheat (91.5%), pea (90%) and lupin (91%). Nevertheless, it exhibited the highest PBV (84%), while the lowest score was for wheat (73%). This result might be the outcome of high content of essential amino acid in rapeseed, and sulfur-containing in particular. The level of rapeseed NPPU accounted for 70.5%, while for milk and soy – 78% and 73.5%, respectively. With regard to excellent PBV in human, rapeseed was concluded as great source of proteins of high nutritional properties. Fleddermann et al. (2013) after cross-over, randomized, double-blind study on 28 male subjects, also stated that rapeseed protein products possess high dietary value and exhibit efficiency similar to soy proteins, with regard to postprandial amino acids response.

Recent studies performed by Zhang, Zhang, et al. (2020) showed that protein solubility of rapeseed meal has an impact on standardized ileal amino acid digestibility in Pekin ducks. In this research, 1168 ducks (14 days posthatch) were subjected to 23 diet treatments for 18 days. The experimental set included one basal diet and 22 rapeseed meal diets consisted of 15% of rapeseed meal and 85% of basal diet. The study showed that standardized ileal digestibility of essential and non-essential amino acids in all meal samples differs significantly, except for leucine and tyrosine. Significant differences were observed also in amino acid composition, proximate nutrients level and antinutritional compounds content with protein solubility varying from 15.06% to 98.08%. Protein solubility was found to be associated with the level of crude fiber, neutral detergent fiber, acid detergent fiber, glucosinolates and isothiocyanates in rapeseed meal. This showed that protein solubility can be one of the factor that can be used to assess rapeseed meal quality.

Since proteins can be modified in order to improve their characteristic (for example protein hydrolysates can be more digestible and less allergenic), Canistro et al. (2017) provided first safety assessment report on rapeseed protein hydrolysates in murinal animal model. In vitro digestibility, metabolic effect and toxicity were investigated. The study showed that hydrolysates were well tolerated and good palatability was also suggested. Investigated factors, such as body weight or blood parameters were similar to the control. In addition, no adverse effects on renal and hepatic drug metabolism enzymes were observed. This research opened a new possibilities in rapeseed protein processing for food and feed purposes.

Different protein modification was also applied by Pan et al. (2020) who proved that in vitro protein digestibility (IVPD) of napin fraction could be improved by means of ultrasound-assisted physical mixing. They also stated that protein digestibility strongly depended on molecules structure, such as disulfide bond content. In napins, these bonds stabilize their structure, but also inhibit digestion in humans. Applied ultrasounds treatment can change secondary structure of protein by decreasing β -sheet content and disulfide bonds content, thus increase digestibility. However, further studies have to be conducted to determine whether this method reduce different protein nutrients or alter nutritional value.

Allergenicity

In recent 15–20 years, a rapid growth of allergic diseases, generated by food products allergens, is observed. It has been estimated that in developed countries 1.5–2% of adults and 5–8% of children are suffering from food allergies. The significant group of food allergens are plant-derived allergens, including 2S albumins, also present in *Brassicaceae* seeds. The characteristic tripartite and quaternary structure of these proteins lead to their high resistance to chemical, thermal and enzymatic denaturation, which is the direct cause of their allergenicity (Czarnecka and Koziołkiewicz 2007; Wanasundara 2011). Mustard, which belong to *Brassicaceae* family, is considered to be one of the most significant food allergen. Generally no fatal-anaphylactic reactions to its seeds have been reported, however they are claimed to be involved in some allergic responses, for example baker's asthma and celiac disease induction. Homologous to mustard proteins, 2S rapeseed albumins were also found to have potential allergenic properties (Lee, Hefle, and Taylor 2008; Monsalve, Villalba, and Rodríguez 2001; Wanasundara 2011). It was presented that *Brassica napus* allergens, named BnIa/BnIb and Bra n I (BnIII) show cross-activity with other *Brassica* genus 2S albumins (Moreno and Clemente 2008). Frigerio et al. (2019) also claimed that mustard plants such as *Brassica juncea* or *Brassica nigra* exhibited cross-reactivity with *Brassica napus*. Poikonen et al. (2008) have concluded that children with atopic dermatitis sensitized to *Brassica napus* and *Brassica rapa* have suffered from allergic rhinitis, asthma and sensitizations to pollens and foods more often than control group. However, it has been pointed out that cruciferins might also potentially possess allergenic properties (Puumalainen et al. 2015). In recent study, L'Hocine, Pitre, and Achouri (2019)

confirmed the presence of major mustard allergens in mustard seeds, which were Sin a 1, Bra j 1 (albumins 2S) and Sin a 2 (globulins 11S). They also found new IgE-binding protein bands in *Brassica juncea* and *Sinapis alba* varieties – β -glucosidase, oleosin, glutathione-S transferase proteins and encolase. According to Puumalainen et al. (2015), both napins and cruciferins might occur in cold-press rapeseed oil, still further research should be carried out to evaluate the clinical significance of the exposure to such low content of proteins in this material. According to European Commission implementing decision, as rapeseed protein can cause allergic reaction to individuals sensitized to mustard and its derivatives, food products containing these proteins have to be adequately labeled (European Commission 2014). However, Fiocchi et al. (2016) in their studies stated that precautionary labeling of food containing rapeseed as potentially cross-reactive might not be accurate, as cross-reaction between mustard and rapeseed occurs only in theory, but has never been described in effect. Researchers stated that this could be confusing for both diet professional and consumers.

Functionality

Functional properties of proteins play very important role with regard to their applications in food formulations. Proteins not only contribute nutritional value to foods, they also stabilize their structure, impact on bioavailability of different compounds and influence foods hedonic features, making them pleasurable to eat. Obviously in certain cases, proteins might also evince undesirable effects, so it is crucial to adopt comprehensive approach to this subject. Protein of various origins can exhibit different functional properties. These properties depend in turn on different aspects, such as production process and application conditions. (Nadathur, Wanasundara, and Scanlin 2017; Foegeding 2015; Foegeding and Davis 2011). In Table 3, functional properties along with affecting them physiochemical features, along with examples of foods, are presented.

Functional properties of proteins may be affected by factors, which can be divided into three main groups: intrinsic factors, processing treatments and application conditions. In Table 4, different factors important for functionality tests are presented (Hall 1996).

According to Ostrowska, Kozłowska, Rachwał-Rosiak, et al. (2018) rapeseed protein isolate possess high potential to be used as functional food component due to its functional properties, such as solubility, emulsifying and foaming capacities.

Solubility

Solubility is the key property for protein dispersions and is strongly associated with other functional properties, such as emulsifying and foaming features, or gelation (Ostrowska, Kozłowska, Rachwał-Rosiak, et al. 2018). Numerous studies on rapeseed protein isolates have showed that cruciferin and napin exhibit different solubility behavior that are dependent on pH values, temperatures, and salt levels (Wanasundara et al. 2016). According to Li et al. (2020), solubility (as well

Table 3. Functional properties of proteins along with their mechanisms and examples of food systems.

Functionality	Mechanisms/Physicochemical features	Food systems
Solubility	Hydrophilicity, hydrogen bonding, surface residues ionization and charge	Milk, nondairy milk, beverages rich in protein
Emulsification	Hydrophilicity and hydrophobicity, film formation and adsorption in oil-water interface	Nondairy milk, cakes, dressings, desserts, comminuted meat
Foaming	Hydrophilicity and hydrophobicity, film formation and adsorption in air-water interface	Cakes, dessert, mousses, ice cream, toppings
Heat – induced gelation	Thermal aggregation, formation of network, immobilization and entrapment of water	Puddings, bakery and emulsified meat products
Water binding	Ionic hydration, hydrogen bonding	Meat (low-fat) and bakery products, comminuted meat
Flavor and fat binding	Entrapment, hydrophobic bonding	Beverages rich in protein, gravies and sauces, bakery products, emulsified meats, flavored milks
Viscosity	Hydrogen bonding, hydrodynamic shape and size	Dressings and gravies, desserts, soups
Elasticity	Disulfide cross-linking, hydrophobic bonding	Bakery (leavened), meat and extruded products
Adhesion and cohesion	Hydrogen bonding, ionic bonding, hydrophobic bonding	Noodles, pasta, bakery products, extruded snacks, emulsified meats

Source: Nadathur, Wanasundara, and Scanlin (2017).

Table 4. Common variables relevant to functional properties.

Functionality	Variables	Examples of other variables
Solubility	pH, temperature, time, mixing method, centrifugation speed,	Mixing times and centrifugation varied
Emulsion capacity and stability	pH, time, mixing method	Measurements of stability over a broad variety of times
Gelation	pH, temperature, time	Often used additives, heating time varied
Water and oil binding or adsorption	pH, time, mixing method, centrifugation speed	Type of oil, mixing times and centrifugation varied
Viscosity	Time	–

Source: Hall (1996).

as other functionalities) is also strongly depended on molecular structure of protein. Their studies showed that alteration of rapeseed protein isolate secondary and tertiary structure caused by ultrasonic treatment directly affected solubility. Exposure to ultrasound conditions may result in that the spatial and molecular structure of proteins can be altered by destroying the bonds formed by electrostatic forces, or hydrogen bonds, with the effect of increasing unfolding of the protein chains. Also, FTIR analysis of spatial structure showed that β -turn and β -sheet increased, whereas random coil and α -helix decreased. Overall, applied modification improved protein solubility.

Wanasundara et al. (2012) demonstrated that in strong acidic conditions napins are soluble in contrast to cruciferins while in strong alkaline solutions both proteins remain in solution. Overall, the addition of NaCl and CaCl₂ improved solubility of tested seed storage proteins. Solubility of cruciferin and napin was also assessed in two comprehensive articles by Cheung, Wanasundara, and Nickerson (2015) and Cheung, Wanasundara, and Nickerson (2014) as the function of NaCl concentration (0 mM, 50 mM, 100 mM) and pH value (3.0, 5.0, 7.0). For both proteins, analysis found the primary effects of pH value and NaCl content to be significant. Interestingly for napins, associated interaction between these variables was not found.

With regard to cruciferins, solubility was the highest at pH 3.0 – approximately 91%, regardless of the NaCl level. At pH 5.0 and 7.0 the lowest solubility was at NaCl concentration of 0 mM, 10.7% at pH 5.0 and 15.7% at pH 7.0, but was increased significantly by the addition of NaCl – at 50 mM, 77.4% and 86.6% respectively and at 100 mM, 88.2% and 90.4% respectively. These results indicate that NaCl is

capable of exerting strong salting-in effect on cruciferin protein isolate. Effect of salt addition on solubility may be explained by enhanced interactions between protein and water mediated by sodium cations. With no NaCl added, cruciferin possessed high solubility only at pH 3.0, as cruciferins remained dispersed thanks to sufficient electrostatic repulsion. However, at pH 5.0 and 7.0 these forces were not adequate, which led to protein aggregation. Worth notice is that with addition of salt much above 100 mM, salting-in effect is observed when pH is maintained below isoelectric point of protein, whereas at pH values above isoelectric point it may cause salting-out of protein. It was also pointed out that protein concentration have impact on solubility – lower solubility could be presumably attributed to higher concentration of protein (Cheung, Wanasundara, and Nickerson 2014). That phenomenon may be explain by increased protein aggregation at high concentration. In that conditions solubility constant of a product may be exceeded (Vojdani 1996). Lower solubility could be also caused by increasing amount of protein-protein interactions (Cheung, Wanasundara, and Nickerson 2014).

In general, solubility of napin protein isolate was high and remained well above 90% in all conditions tested, and was merely impacted by the changes in NaCl concentration. At pH 3.0 and 5.0 solubility was similar, 98.2% and 99.2%, respectively, but slight decrease was observed at pH 7.0 (96.3%). Examined proteins were entirely soluble at NaCl concentration of 50 mM. With the addition of 0 mM and 100 mM NaCl, the result were similar, 96.8% and 96.5%, respectively. The findings were consistent with results of other studies and provide solid evidence that napin protein isolate in native form is entirely soluble in wide range of pH

values. The authors pointed out that napins exhibit extremely basic isoelectric point (above pH 10) along with hydrophilic nature and conclude that high solubility should be related to high surface charge and low hydrophobicity of the native napin molecule. High solubility may contribute to good emulsifying properties, as proteins are able to diffuse to the oil-water interface and diminish the interfacial tension (Cheung, Wanasundara, and Nickerson 2015).

In their study, Karaca, Low, and Nickerson (2011) pointed out that method of production of protein isolates exerted great impact on protein solubility, as they measured that solubility of canola protein isolate obtained by salt extraction was approximately 80%, whereas isolate obtained by isoelectric precipitation – below 5%.

Sibt-e-Abbas et al. (2020) investigated nitrogen solubility of canola protein isolates obtained by alkaline extraction and subsequent acidic precipitation. The solubility strongly depended on pH value. The lowest solubility (7.32%) was observed at pH = 4, while the highest (62.51%) – at pH = 12. The lowest solubility at pH = 4 may be explained by the proximity to the isoelectric point; at pH = 2, this parameter increased to 40.42%. High solubility at alkaline conditions can be caused by proteins dissociation and disaggregation.

Kalaydzhiev et al. (2020) studied the impact of sequential isoelectric precipitation on solubility of two rapeseed protein isolates obtained from industrial rapeseed meal. Protein isolate $PI_{10.5-2.5}$ was obtained by alkaline extraction (pH = 12) and precipitation from pH 10.5 to 2.5, whereas protein isolate $PI_{2.5-8.5}$ was subsequently precipitated from pH 2.5 to 8.5. The study showed that solubility depended on pH value and salt addition, however these two isolates had similar solubility profile with almost complete solubility at pH ≥ 7 . Below that value, solubility was lower and differed strongly between the isolates. $PI_{10.5-2.5}$ had higher solubility ranged from 41.75% (pH 4.5) to 65.13% (pH 6.5), whereas $PI_{2.5-8.5}$ exhibited almost twice less solubility in this particular conditions. This was explained by that these isolates possessed different fractional profile. $PI_{10.5-2.5}$ had probably more basic proteins with the isoelectric point around pH 9–10.5. This isolate was presumably better hydrated in acidic pH, due to positive charge of the molecules. In contrast, $PI_{2.5-8.5}$ was supposed to had more proteins with acidic isoelectric points. In case of NaCl supplementation, it was observed that the addition of salt at 0.03 and 0.25 M concentration increased the solubility of $PI_{2.5-8.5}$ and decreased the solubility of $PI_{10.5-2.5}$, when pH value was up to 5.5. At pH > 7 , addition of salt significantly enhanced the solubility of $PI_{2.5-8.5}$, whereas for $PI_{10.5-2.5}$ no effect of NaCl addition was observed.

Emulsifying properties

Emulsifying properties of cruciferin protein isolate and napin protein isolate were investigated also by Cheung, Wanasundara, and Nickerson (2015) and Cheung, Wanasundara, and Nickerson (2014) under the influence of NaCl concentration (0 mM, 50 mM, 100 mM) and pH value (3.0, 5.0, 7.0). They determined emulsifying activity indices (EAI) and emulsifying

stability indices (ESI) of the protein isolates. The EAI relates to the interfacial area which is covered by an emulsifier or surfactant (for example protein) during emulsification process. The EAI value may also serve as indicator of surface activity of proteins. The ESI provides an assessment of stability of emulsion. In regard to EAI, for both cruciferin and napin, analysis showed that the primary effects of pH value, NaCl content and associated interaction between these factors were all significant. The same tendency was observed for napin in relation to ESI values, whereas for cruciferin only salt effect was found to be significant.

For cruciferin, at pH = 3.0, EAI values ranged from 18.8 m²/g to 19.4 m²/g, thus no significant effect of NaCl level was observed, as opposed to pH 5.0 and 7.0 that EAI values declined, as the content of salt increased. With no salt addition, EAI equaled 21.1 m²/g and 14.9 m²/g, in pH 5.0 and 7.0, respectively and at 100 mM salt EAI decreased to 12.8 m²/g and 5.2 m²/g. This particular study showed positive correlation between emulsifying activity and hydrophobicity of the surface. High surface hydrophobicity supports protein arrangement in the interface, which in turn leads to reduction of the interfacial tension. The highest ESI values were measured at 0 mM NaCl content. Overall, the ESI declined from approximately 15.1 min (0 mM NaCl), to approximately 11.6 min (50 mM NaCl) and approximately 12.0 min (100 mM NaCl). These results were found to be associated with charge of the surface. The increased salt level diminishes surface charge, resulting in decrease of the electrostatic repulsive forces, which leads to flocculation and coalescence of the droplets. Negative correlation between solubility and ESI was also observed (Cheung, Wanasundara, and Nickerson 2014).

Overall, emulsifying properties of napin protein isolate were similar at pH 3.0 and 5.0, but lower at pH 7.0 and the EAI values were equal 19.4 m²/g, 18.7 m²/g and 12.8 m²/g, respectively. The salt effect exhibited similar trend at pH 3.0 and pH 7.0. Measured values were the highest at 50 mM NaCl concentration, and the lowest with the addition of 100 mM NaCl. At no salt addition, only slight increase was observed as compared to 100 mM salt. At pH 5.0, EAI reached the highest value at 0 mM NaCl and showed diminishing trend as the content of salt increased. The authors claimed that high emulsifying capabilities are connected with high charge of the surface in combination with good solubility of the protein. However, in this particular study, napin protein isolate exhibited very high solubility in all conditions tested, so it was pointed out that other variables also impact emulsification process. Stability of the tested emulsion was the highest in the absence of salt and ranged from 16.0 to 16.8 min. At pH 3.0, as the concentration of salt increased, ESI values were reduced. With 100 mM NaCl, ESI declined to 12.6 min. At pH 5.0 and 7.0 the lowest values were measured for 50 mM NaCl. It was stated that high emulsion stability is correlated with high surface charge, as it can provide stronger electrostatic repulsive forces between emulsion droplets. This effect might be reduced by increased salt concentration, due to aggregation or flocculation of the droplets. The authors also concluded that napin protein isolate might be used in food and beverages, especially in those

requiring acidic pH and addition of lipid components (Cheung, Wanasundara, and Nickerson 2015). Noteworthy, Foegeding (2015) pointed out that from the general point of view beverages with proteins as ingredients can be considered as sols, in where aggregates of proteins are solid dispersed phase.

The emulsifying properties were also researched by Tan, Mailer, Blanchard, and Agboola (2014), where canola protein isolates: globulin, albumin and canola protein isolate, were examined. They obtained albumin and globulin fractions by the Osborne method with modifications. Canola meal was subjected to deionized water extraction and supernatant was filtrated and concentrated in order to receive albumin fraction. The residue after extraction was extracted with NaCl and dialyzed with deionized water to produce globulin fraction. Canola protein isolate was obtained from canola meal by direct basic extraction and subsequent acidic precipitation. It was concluded that globulin fraction possessed the best emulsifying capabilities and the smallest droplet size, as compared to canola protein isolate and albumin fraction. The EAI of mentioned products was estimated under the influence of pH that ranged from 4.0 to 9.0. The study showed that EAI of albumin fraction and canola protein isolate increased, as pH value increased, whereas EAI of the globulin fraction remained comparatively constant irrespective of pH value. At all pH values tested, globulin fraction reached the highest results and canola protein isolate the lowest, with albumin fraction in between these two. With regard to canola protein isolate, its poor emulsifying activity may be caused by the presence of polypeptides with high molecular weight (likely above 250 kDa), whereas globulin and albumin fractions consist of polypeptides with ≤ 60 kDa. Moreover, canola protein isolate includes disulfide bonds which may have negative impact on EAI via impairing the ability to unfold the polypeptide structures. In this study, emulsifying capacity (EC) was also evaluated. This measurement allow to assess the volume of oil which is emulsified by a gram of protein. The study showed that EC differed significantly depending on pH value (4.0, 7.0 and 9.0) for all samples. In each pH, globulin fraction possessed the highest EC, and canola protein isolate – the lowest. The results of globulin fraction ranged from 1490 to 1734 ml/g, of albumin fraction – 887–1001 ml/g and of canola protein isolate – 461–792 ml/g. High and relatively constant EC of globulin fraction indicates that it may be used in various food applications. The analysis of average droplet size of protein-stabilized emulsions showed that in all pH values (4.0, 7.0 and 9.0) globulin fractions had the smallest droplet size and canola protein isolate – the biggest, as compared to albumin fraction. This results confirmed low emulsifying properties of canola protein isolate and indicated that coalescence might occur immediately after emulsion formation. Altogether, results of EAI, EC and average droplet size were found to be with agreement with each other. In this research, emulsion stability was also assessed by evaluation of changes after physical separation and in droplet size. That study showed that fractionated canola protein exhibited high emulsion stability under most conditions (except canola

protein isolate at pH 4.0) and no significant changes were observed in droplet size while 7 days-long emulsion storage. However, the majority of emulsions, (except canola protein isolate at pH 9.0) separated into serum layers and emulsion and this phenomenon is called creaming.

Karaca, Low, and Nickerson (2011) in their study examined, among others, EC, EAI and ESI of canola protein isolates obtained by salt extraction and isoelectric precipitation. The EC measurements, expressed as gram of homogenized oil per gram of protein (before inversion), showed that results for canola protein isolates ranged from 502.2 g/g (isoelectric precipitation) to 528.9 g/g (salt extraction). The emulsifying activity indices differed significantly with regard to production method – for salt precipitation EAI was over twice as high ($35.1 \text{ m}^2/\text{g}$), than for isoelectric precipitation ($15.0 \text{ m}^2/\text{g}$). The ESI of canola protein isolate produced by salt extraction was also higher when compared to the one obtained by isoelectric precipitation.

Emulsifying capacity of protein rich extract obtained from different Scandinavian varieties and by the use of different process conditions was investigated by Östbring, Tullberg, Burri, et al. (2019). The study showed that heat treatment during recovery process significantly decreased the ability of rapeseed protein to stabilize emulsion droplets' oil-water interface. Protein extract obtained from Lyside variety possessed the highest emulsifying capacity of all varieties tested without heat treatment. When the heat was applied, proteins extracted from Mixed and Alegria varieties possessed the highest ability to stabilize the oil-water interface. What is more, droplet size of the emulsion was reduced with the increase of protein concentration for all varieties and recovery conditions. The researchers also indicated that among rapeseed proteins, it is particularly oleosin that exhibits strongest emulsifying abilities.

Emulsifying capacity (EC) and stability (ES) was examined also by Sibte-Abbas et al. (2020). The study showed that canola protein isolate had $\text{EC} = 65.40\%$ and $\text{ES} = 71.97\%$. According to the authors, relatively low EC might have been caused by decreased amount of hydrophobic residues on the protein surface, which affected in diffusion of oil droplets to the continuous phase.

Östbring, Malmqvist, Nilsson, et al. (2019) proved that emulsifying properties are strongly depended on starting material and technological process (exposure to heat). When no heat was applied, proteins recovered from cold-pressed cake possessed the highest emulsifying properties, in comparison to samples obtained from hot-pressed and solvent-extracted meal. With the use of heat, emulsion stability was significantly lower for all protein samples. The researchers concluded that heat treatment had great impact on emulsifying properties and particularly when applied in the recovery process, rather than in the oil processing. That could be explained by the fact that during recovery process, proteins are solubilized and thus more sensitive to heat, whereas during oil extraction they are protected by seed matrix. Noteworthy, heat treatment can have positive effect on emulsifying capabilities for some proteins. It was explained that it is related to the exposure level of hydrophobic

regions. It was also stated that heating could modify secondary and tertiary structure of proteins, causing aggregation and increase in hydrophobicity, which disturb the balance between hydrophilic and hydrophobic groups on the molecules surface. Wu et al. (2020) pointed out that emulsifying properties depended on proteins structure. They observed using CD spectroscopy that stability of emulsion positively correlated with α -helix proportion. This indicated that environment agents have impact on protein hydrogen bonds and thus on emulsion stability.

Foaming properties

Proteins are able to form foams due to their amphiphilic structure, as they possess the ability to reduce surface tension at air – water interface, where they are adsorbed. This process occurs in three steps: diffusion to the subsurface, adsorption and unfolding at the interface. Foams are also stabilized due to viscoelastic film of proteins around air bubbles (Malabat et al. 2001). Stable foams have to possess the ability to oppose mechanical and gravitational stresses (Gerzhova et al. 2015b). Interfacial activity in air – protein dispersion of 2S and 11S rapeseed proteins varies due to their different molecular weights. Pure (93%) napin proteins exhibited excellent ability to create stable foams, as compared to cruciferin proteins. However, different studies showed that 11S proteins, as well as 2S protein possessed great foaming capabilities (Wanasundara et al. 2016). Gerzhova et al. (2015b) investigated foam capacity and stability of canola protein isolate under different pH values (4.0, 7.0, 9.0). The research showed that the best foaming capacity was at pH 9.0 (66.91%). Slight decrease was observed at pH 7.0 (57.83%) and at pH 3.0 foam capacity was significantly lower (19.62%). Foam stability was measured within 60 min and gradual decline was observed which indicated that foam was not stable. Yet, the highest foam stability was obtained at pH 9.0, and the lowest – at pH 4.0, as compared to pH 7.0. Solubility and hydrophobicity were indicated as important parameter influencing foam capabilities. It was pointed out that foam capacity is related to good balance of hydrophobic and hydrophilic groups which facilitate air bubbles stability. However, insoluble proteins can also be involved in this process, as they might prevent air bubbles coalescence. It was indicated that hydrophobicity might be more correlated with foam capacity, than solubility. The authors referred to different research which showed that regardless of solubility, proteins with low foam capacities exhibited low hydrophobicity. It is noteworthy that generally high foam capacity is not correlated with high foam stability, as molecular features needed in these processes are different. For example, high film yield and viscosity of the surface might have adverse effect on foam formation, whereas they are needed for stabilization process.

Sibt-e-Abbas et al. (2020) also determined foaming capacity and stability of canola protein isolate. The research showed relatively low foaming capacity (12.29 ml). According to the authors, it was attributed to denaturation of disulfide bonds,

which effected in lower flexibility. This could have been caused by processing conditions applied for protein isolation.

Gelation

Gelling properties of proteins are very desirable in food applications, as gel forms are base of many food structures, for example jellies, cheeses, yogurts, sausages, tofu, or puddings. Gelation is a process of aggregation of proteins that form matrix or well-organized tertiary network which is able to hold great amount of water, due to good balance between repulsive and attractive forces. In this phenomenon, two steps are distinguished: fragmentary denaturation associated with changes in protein conformation and subsequent aggregation of denatured molecules (Matsumura and Mori 1996).

According to Yang et al. (2014), heating temperature and pH are crucial variables in formation of gels with canola proteins. They studied cruciferin and napin fractions (15% w/v) in wide range of temperature (80–120 °C) and pH (5.0–11.0). The results showed that cruciferin and napin isolates possessed distinct gel and thermal features, as they are characterized with different composition of amino acids and molecular sizes. Overall, cruciferin exhibited better gel properties than napin. Notwithstanding the temperature, napin produced weak gels at pH 5.0, which might have been caused by fragmentary unfolding of molecules at acidic conditions. At pH 7.0, slight enhancement of gels compressive stress was observed as temperature increased. The gels springiness was the highest at 120 °C, at pH 5.0 and 7.0, while at pH 3.0 remained relatively constant. The mechanical features of napin heat-induced gels were the highest at 120 °C and pH 9.0. It might be related to disulfide bridges breaking at basic environment, that facilitate non-covalent and covalent interplays. With regard to cruciferin gel, measured compressive stress at pH 5.0 was not dependent on temperature. At pH 7.0 and 9.0 the compressive stress value became higher, as temperature rose to 110 °C. The strongest gels were formed at pH 11.0. Generally, napin and cruciferin build elastic and strong gels at high temperatures and basic conditions. The author pointed out that this environment might not fit in food application, however gels can be used in various non-food applications, such as biosensors, tissues engineering, or medical dressings.

Different studies investigated gelling properties of canola protein isolates under different pH conditions (pH 3.0–9.0). The study of Kim, Varankovich, and Nickerson (2016) showed that stronger gel was formed at pH 9.0, than at pH 7.0 and at pH 3.0 the gelation process did not occur. Differential scanning calorimetry measured the denaturation temperature which was approximately 78 °C at pH 5.0 and approximately 87 °C at higher pH values (7.0/9.0). Temperature of gelation was found to be approximately 82–86 °C irrespective of pH. Measured gel density enhanced with increasing pH value. Overall, canola protein isolate exhibited great gelling features. However, the strongest gels were formed under basic conditions and optimal value for this process was pH 9.0, which might be limiting factor for food applications

Canola protein isolate and its globulin and albumin fractions were examined regarding to salt level and pH effect by Tan, Mailer, Blanchard, Agboola, and Day (2014). Generally, gel properties, such as elasticity, firmness, fracture stress, microstructure density and overall deformation resistance improved, as pH values increased. The addition of NaCl had negative impact on globulin and albumin gel features, such as firmness, fracture stress and overall deformation resistance, but improved these properties in canola protein isolate gels. However, elasticity of all canola protein products was at the same level, regardless of NaCl concentration. To sum up, globulins performed better in gelling tests, as compared to albumins and canola protein isolate, which is in agreement with other studies. According to this article, canola proteins might be used as a gelling agent in such food products as sausages, soups, sauces and jams, as these applications needed to possess thickened or gel – like structure, but not necessarily good clarity, which could be difficult to reach, because of cloudiness and coloration of protein solutions.

Sibt-e-Abbas et al. (2020) also studied gelling abilities of rapeseed proteins, by determining least gelation concentration. The research showed that at 10% concentration some gelling ability of canola protein isolate was observed (partial gel was formed), and at concentration 14% strong and stable gel was obtained.

Anti-nutritional and other compounds

Canola meal is characterized by well-balanced amino acid composition, yet it contains some undesirable anti-nutritional factors such as glucosinolates, phenolic compounds including sinapine and condensed tannins, phytates, or fiber (Aider and Barbana 2011; Tan et al. 2011b; Mailer et al. 2008). Anti-nutritional compounds are plants' secondary metabolites which are formed during different metabolic processes as a side products. They demonstrate high biological activity and play various roles in plant, for example protective, antifungal, antimicrobial, pesticidal, insecticidal or storage (Gemede and Ratta 2014; Soetan 2008). According to CCC (2015) typical canola meal (12% moisture basis) includes among others 1.0% sinapine, 2.3% phytic acid, 4.2 $\mu\text{mol/g}$ glucosinolates and 32.4% total dietary fiber. Despite of having potentially profitable health effects, for example diminishing cancer risk, while occurring at very low levels, these compounds are still considered to be the main disadvantage of protein use for food applications, and many of them have to be removed or reduced to an appropriate amount, in order to receive applicable to human food, protein product (Tan et al. 2011b; Wanasundara et al. 2017). However, it has been stated that during the technological processes in order to obtain canola proteins, up to 100% of tannic acid, 92% of phytic acid and 95% of glucosinolates can be eliminated (GRAS 2016). Hald et al. (2019) conducted a breakthrough research and confirmed for the first time a molecule which plays a key role in undesirable bitter taste of rapeseed protein isolates – kaempferol 3-O-(2'''-O-Sinapoyl- β -sophoroside). This component had low threshold (3.4 $\mu\text{mol/l}$) and above this concentration exhibited unpleasant bitter taste. Noteworthy cruciferin-rich

protein isolate, assessed as having the most intensive bitter off- taste, had significantly higher concentration of this compound (475.6 $\mu\text{mol/kg}$) in comparison to napin-rich protein isolate (32.9 $\mu\text{mol/kg}$) and rapeseed meal (32.0 $\mu\text{mol/kg}$). This key findings can contribute to developing new technologies which will enable to obtain rapeseed protein isolates with desirable taste profile.

Phenolics

Phenolics are reported as compounds, which evince such profitable effects as anti-allergic, anti-inflammatory, anti-atherogenic, anti-thrombotic, vasodilatory, anti-microbial and cardio-protective features (Szydłowska-Czerniak 2013). Phenolic acids are predominant phenolic compounds present in rapeseed. They are found as a free constituents but also in the glycosidic, esterified or insoluble-bound forms. These components are identified to be derivatives of cinnamic and benzoic acid (Amarowicz, Kolodziejczyk, and Pegg 2003). The most dominant phenolic constituent in canola is the sinapic acid choline ester named sinapine – approximately 80%, while the prominent free phenolic acid is sinapic acid – over 73% (Wanasundara et al. 2016; Khattab et al. 2010). The substantial phenolics of canola is also sinapoyl glucose and vinylsyringol, which is claimed to possess antioxidant activity in canola oil polar fractions. Rapeseed and canola may also contain such acids as vanillic, *p*-hydroxybenzoic, *p*-coumaric, caffeic, ferulic, chlorogenic, syringic, protocatechuic and gentisic (Khattab et al. 2010). In rapeseed, soluble and insoluble tannins are also present. Rapeseed meal contains up to 6% tannins (Amarowicz, Naczek, and Shahidi 2000). The aqueous media, pH and salt used to obtain protein products, may lead to development of complexes between phenolics and proteins, which is tough to prevent (Wanasundara et al. 2016). At basic pH values, phenolic compounds are effortless to extract from canola meal, therefore obtained protein may be of dark color and inexpedient taste (Xu and Diosady 2000). Complexation between phenolic compounds and rapeseed proteins can be reversible or irreversible and may reduce nutritional availability of proteins (Vuorela 2005). Reported values of rapeseed isolates' phenolics present as follows. Puratein[®] possesses 0.40% and Supertein[™] – 0.26% of total phenolic acids, expressed as sum of sinapic acid, which constitute over 90%, *p*-coumaric acid and ferulic acid, as others are not detected (GRAS 2010). Isolexx[™] is characterized with total phenolics content in amount of 0.14% (expressed as gallic acid) and possesses 0.005% of condensed tannins and 0.08% of derivatives of sinapic acid (GRAS 2011). Total phenolics content, expressed as sinapic acid, in 5 representative and independent batches of CanolaPRO[™] ranges from 600 to 881 ppm (GRAS 2016). Rapeseed protein isolate obtained by NFB's technology possesses 0.02% phenolic compounds (Ostrowska, Kozłowska, Rachwał-Rosiak, et al. 2018).

Phytates

myo-Inositol (1, 2, 3, 4, 5, 6) hexakisphosphoric acid, known as phytic acid, or phytate, when in the form of a salt with calcium, magnesium and potassium, is naturally occurring compound, which stores phosphorus in plants (Greiner, Konietzny, and Jany 2006; Wanasundara et al. 2017; GRAS 2011). Canola meal contains phytates in IP6 (*myo*-inositol hexakisphosphate) and IP5 (*myo*-inositol pentakisphosphate) forms, which account for 1.5–2.1% and 0.1–0.2%, respectively (Wanasundara et al. 2016; Wanasundara et al. 2017). Phytates can bind with many metal cations (divalent and trivalent) and this ability is the reason for the main concern about their prevalence in human diet, because it can cause adverse effects of mineral absorption. Decreased availability of such minerals as zinc, iron, calcium, magnesium, manganese and copper may lead to their deficiencies. In addition, phytates are able to complex with proteins in wide range of pH value, both acidic and basic. These interactions are supposed to diminish in vitro protein digestibility. However, several beneficial effects of phytates were reported, including prevention against kidney stones, cariosity, diabetes, coronary disease, or atherosclerosis (Greiner, Konietzny, and Jany 2006). Furthermore, it has been demonstrated that IP6 form displays anticarcinogenic effect via apoptosis induction (Verghese et al. 2006). Following values of phytic acid content in commercial products were reported. Cruciferin – rich Puratein® contains 0.32% and napin – rich Supertein™ – 3.34% of phytic acid (GRAS 2010), which indicates that level of phytates may varies in different protein fraction. Isolexx™ (four representative lots) possesses 0.44–1.14% dw (dry weight) of total phytates (GRAS 2011), whereas in 5 representative and independent batches of CanolaPRO™ phytic acid level accounts for less than 0.14% (GRAS 2016).

Table 5. Carbohydrates in Puratein® and Supertein™, expressed as %.

Carbohydrates	Puratein®	Supertein™
Arabinose	0	0.005
Xylitol	0	0
Xylose	0	0.056
Fructose	0.016	0.034
Mannose	0	0.006
Galactose	0.016	0.027
Glucose	0.032	0.057
Sucrose	0.137	0.467
Maltose	0	0
Isomaltose	0	0
Raffinose	0.009	0.033
Stachiose	0.006	0.257

Source: GRAS (2010).

Level of phytic acid of NFB's rapeseed protein isolate accounts for 0.13% (Ostrowska, Kozłowska, Rachwał-Rosiak, et al. 2018).

Glucosinolates

Glucosinolates are sulfur and nitrogen containing glycosides, which presence in rapeseed was formerly the major concern about utilizing this plant as feed. These compounds were proved to possess some adverse effects, such as decreasing animal performance, or diminishing thyroid activity in animal fetuses and growing animals. What is more, glucosinolates of *Brassica* are recognized to be bitter, and thus responsible for cruciferous vegetables unsavory taste. However, they were also investigated to protect crops, prevent cancer and perform as flavors precursors. Due to breeding programs, glucosinolates content is now on trace levels (Mailer et al. 2008; El-Beltagi and Mohamed 2010; Drewnowski and Gomez-Carneros 2000). According to El-Beltagi and Mohamed (2010), the main glucosinolates found in five different rapeseed cultivars are progoitrin and gluconapin and total glucosinolates content ranges from 4.40 to 5.97 $\mu\text{mol/g dw}$. Total glucosinolates content in Puratein® is reported to be 1.22 $\mu\text{mol/g}$, whereas in Supertein™ – 0.80 $\mu\text{mol/g}$ (GRAS 2010). In four representative lots of Isolexx™ total glucosinolates range from 0.05 to 0.15 $\mu\text{mol/g dw}$ (GRAS 2011) and in 5 representative and independent batches of CanolaPRO™ glucosinolates account for less than 0.1 $\mu\text{mol/g}$ (GRAS 2016).

Carbohydrates and fibre

Canola seeds contain free sugars, as well as fiber fractions, which are primarily presented in the hulls. Insoluble dietary fiber (IDF) fraction is mainly consisted of cellulose and lignins, whereas hemicellulose, pectins, gums mixed β -glucans and mucilage are found in soluble dietary fiber (SDF) fraction (Wanasundara et al. 2016). High level of fiber may decrease nutritional value of the feeds, due to diminishing their digestibility (Mailer et al. 2008). However, dietary fiber possesses well known, beneficial effect on human health, such as preventing from type 2 diabetes or cardiovascular diseases. WHO/FAO stated that daily intake of dietary fiber, preferably from various sources, should be minimum 25 g (Guiné et al. 2016). According to Slominski et al. (2012), conventional (black – seeded) *Brassica napus* canola contains: 0.2% dw monosaccharides (glucose and fructose),

Table 6. Estimated levels of rapeseed protein isolate (CanolaPRO™) in proposed food products for general population (age 4 and above).

Type of products	Max. usage level [% RPI in final food]	gram RPI/serving	% RPI of total protein
Prepared food	10	20	40
Meat analogues	30	20	90
Bakery products	5	5	30
Protein enriched bakery products	30	10	70
Sports nutrition	10	20	90
Weight management	30	20	90
Beverages	5	10	100
Dairy products	5	5	75
Medical nutrition	10	20	100
Elderly nutrition	10	10	75

Source: GRAS (2016).

Table 7. Food applications of rapeseed protein products.

Type of products	Application	Reference
Bakery and confectionery products	• Napin-rich protein isolate (Supertein™) and cruciferin-rich protein isolate (Puratein®) can constitute up to 2% of bakery products, such as bread, rolls, croissants, cakes, brioches, bagels, croutons, muffins, biscuits, crumpets, scones, tortillas, cookies, crepes, donuts, bars, waffles, pancakes etc. • Supertein™ and Puratein® can constitute up to 50% of high protein nutrition bars • Suggested level of Isolexx™ in various bakery products is max. 3% • Isolexx™ can be incorporated up to 30% to nutritional bars • It is found to be feasible to replace eggs in pound cakes even up to 100% by rapeseed protein concentrate, however obtained products might have a little darker color as compared to reference • Canola protein isolate and concentrate can be added in amount of 18% to bread dough as wheat flour proteins replacement without adverse effects on loaf and dough properties • Canola protein isolates and concentrates can be used as rice flour replacement in gluten-free biscuits • Canola proteins can be incorporated to gluten-free bread based on white rice flour up to 6% as they exhibit similar properties to gluten. At this concentration, improvement of technological properties of the dough and bread and increased nutritional value were observed. Supplementation had also beneficial impact on final product's physicochemical features, as well as functional properties of the dough.	GRAS (2010) GRAS (2010) GRAS (2011) GRAS (2011) Von Der Haar et al. (2014) Wanasundara et al. (2016) Gerzhova (2016) Salah, Olkhovarov, and Aider (2019)
Beverages	• Supertein™ and Puratein® can constitute up to 10% of fruit and vegetable juices, for example orange juice or tomato juice, as well as, various flavored beverages • Proposed level of Isolexx™ in beverages and soups is max. 5% • Isolexx™ can be incorporated up to 9% to protein drinks and dry instant milkshakes	GRAS (2010) GRAS (2011) GRAS (2011)
Dairy products	• Supertein™ and Puratein® can constitute up to 5% of wide range dairy products, including cream and whipped cream substitutes, cheese spreads and dairy-based drinks • Supertein™ and Puratein® can constitute up to 60% of egg substitutes and scrambled eggs made from powdered blend • Estimated amount of Isolexx™ in different dairy products is max. 4% • Rapeseed protein isolate can be incorporated to meringue formulations in amount of 3% as egg white substitute	GRAS (2010) GRAS (2010) GRAS (2011) Wanasundara et al. (2016)
Dressings and sauces	• Supertein™ and Puratein® can constitute up to 2% of various types of dressings, for example mayonnaise-type • Mayonnaises prepared with rapeseed protein ingredients as eggs substitutes exhibit similar firmness as regarded to reference • Rapeseed proteins can be used as multifunctional food ingredient that possess emulsifying properties and delay oxidation effect • An innovative mayonnaise-like plant-based emulsion was obtained by controlled extraction of oleosomes (and co-extracted compounds) from rapeseed seeds with no emulsification step and sensory features similar to commercial products	GRAS (2010) Von Der Haar et al. (2014) Östbring, Tullberg, Burri, et al. (2019) Romero-Guzmán et al. (2020)
Flavorings	• Enzymatic hydrolysates from <i>Brassica napus</i> proteins can be used to produce meat-like flavorings. It is suggested to conduct process of meat aroma constitutes generation at lower pH value and lower temperature to form cooked meat odor, or higher temperature to obtain roasted meat odor. The most favored conditions are 160 °C and pH equal 4.0, whereas choosing 180 °C and pH equal 8.0 implicates burnt-like characteristics	Guo, Tian, and Small (2010)
Meat products	• Supertein™ and Puratein® can constitute up to 2% of processed meat products, including frankfurters, bolognas, different types of sausages, hot dogs, hams, meat-based soups etc. • Napin and casein assemblies are supposed to have the ability to substitute pure casein in sausage batters or sauces • Rapeseed protein hydrolysate can replace casein, added in amount of 2%, in sausage formulation, which possesses good texture, distinctive aroma and improved taste • Optimal concentration of rapeseed protein ingredients in sausages is found to be no more than 2%, due to possible occurrence of undesirable off-flavors and unaccepted color	GRAS (2010) Schwartz et al. (2015) Yoshie-Stark et al. (2006) Von Der Haar et al. (2014)
Snacks	• Isolexx™ can be incorporated to various snack foods, including crackers, candy ingredients and snack chips, in max. amount of 20%	GRAS (2011)
Vegetarian food	• Isolexx™ can be added to vegetarian foods and meat analogues in amount of max. 20%	GRAS (2011)

Table 8. Bioactive properties of canola proteins and peptides.

Bioactivity	Study conclusion	Reference
Antihypertensive and angiotensin I-converting enzyme (ACE) inhibiting	• Rapeseed protein hydrolysates are claimed to be valuable source of ACE inhibitory peptides • Two ACE-inhibitory peptides Val-Ser-Val and Phe-Leu are presented in napin and cruciferin primary structure • Alcalase hydrolysate, which displays ACE-inhibitory effect, remains stability while dealing with human peptic fluids • Peptide Arg-Ile-Tyr named rapakinin, obtained from rapeseed, possesses antihypertensive activity and presents vasorelaxing effect in spontaneously hypertensive rats' the mesenteric artery • Alcalase hydrolysate are stated to have potential uses in food therapeutic product, as well as, to prevent and treat hypertension	Pedroche et al. (2004) Wu, Aluko, and Muir (2008) Mäkinen et al. (2012) Yamada et al. (2010) Wu, Aluko, and Muir (2009)
Anorexigenic	• Tripeptide Arg-Ile-Tyr obtained from rapeseed protein suppresses food intake gastric emptying by cholecystokinin release stimulation, after oral application in mice	Marczak et al. (2006)
Anticancer	• Rapeseed peptide shows inhibition of human cervical carcinoma (Hela) cell proliferation and growth and thus might be considered as potential antitumor compound	Xue et al. (2010)
Antidiabetic	• Rapeseed protein shows inhibitive effect on early initiation of insulin resistance in rats fed with diet rich in saturated fat and sucrose	Mariotti et al. (2008)
Antioxidative	• Rapeseed protein hydrolysates exhibit scavenging activity against DPPH, superoxide and hydroxyl radicals • Peptides obtain from rapeseed meal protein hydrolysates inhibits ferrous-induced phosphotidyl choline oxidation • Antioxidant properties of protein hydrolysates obtain in enzymatic pathway is suggested to depend on composition, thus can be modified by altering the enzyme type during the hydrolysis process • Amino acid composition does not have impact on antioxidant activity of canola protein hydrolysates, which suggests higher impact of particular sequences of amino acids in peptide chain	Pan, Jiang, and Pan (2011) Wanasundara et al. (2016) Cumby et al. (2008) Alashi et al. (2014)
Antifungal	• Napin obtained from industrial rapeseed meal exhibits significant activity for <i>Fusarium langsethiae</i> growth suppression	Nioi et al. (2012)
Antithrombotic	• Rapeseed peptides demonstrate inhibitory effects on fibrogen coagulation catalyzed by the thrombin, however it is much lower, as compared to heparin	Zhang, Wang, and Xu (2008)
Antiviral	• In rapeseed protein hydrolysates, HIV protease inhibitors are presented	Yust et al. (2004)
Bile acid binding	• Protein isolate obtained by precipitation possesses higher bile acid binding capacity in comparison to protein isolate obtained by ultrafiltration, probably due to higher amount of fiber	Yoshie-Stark, Wada, and Wäsche (2008)
Cell growing	• Rapeseed protein hydrolysate shows the ability to enhance growth of insect Sf9 cell in serum-free medium • One fraction of rapeseed protein hydrolysate presents significant stimulation of CHO cell growth rate • Rapeseed peptide fraction possesses ability to enhance performance of various industrial animal cell lines, including CHO, improve their serum-free adaptation, as well as, increase the amount of produced IFN-γ	Deparis et al. (2003) Farges-Haddani et al. (2006) Farges et al. (2008)

8.8% dw sucrose, 3.1% dw oligosaccharides (including 0.6% dw raffinose and 2.6% dw stachyose), 0.4% dw starch and 30.1% dw dietary fiber. Carbohydrates profile of Puratein[®] and Supertein[™] is presented in Table 5. Isolexx[™] (four representative lots) possesses from 2.3 to 6.8% dw of carbohydrate and from 0.00 to 0.06% dw of fiber (GRAS 2011). Results of five production batches of CanolaPRO[™] show that isolate contains less than 0.6% w/w fiber and carbohydrates are reported absent (GRAS 2016).

Food applications

Plant-based diet is proven to exert favorable effects on human health, such as lowering risk of diabetes and/or coronary diseases. In addition, processes for production of

plant proteins are cheaper and more friendly to the environment than those based on dairy and meat production (Shahidi 2009; Schwartz et al. 2015). According to Schwartz et al. (2015) rapeseed is one of the most significant sources of plant protein on a global scale. Also Jones (2016) concluded that rapeseed is a promising possible source of proteins which might be used as meat substitutes.

As canola proteins are demonstrated to possess beneficial nutritional and functional value, numerous studies have been conducted in the past decades with the aim to apply them to food products as a replacement of animal – derived proteins. Rapeseed protein isolate can play various roles in food, as emulsifier, binder, foaming and gelling agent, thickener, texturizer or simply as a protein source. Thereby, a wide range of food application with canola proteins, including bakery,

Table 9. Non-food and non-feed uses of canola proteins.

Utilization	Research details	Reference
Adhesive	• Conjugates of canola protein isolate with poly(glycidylmethacrylate) obtained by free radical polymerization exhibit good water resistance and adhesive strength	Wang, Wu, and Bernard (2014)
Carrier and delivery systems	• Calcium-induced cruciferin particles (because of cruciferins' resistance to gastric enzymes), obtained by cold gelation method, could be used as potential carriers of nutraceutical food constituents	Akbari and Wu (2016)
Protein-based films	• The specific characteristics of canola proteins makes them suitable for use as carriers to deliver, protect and encapsulate drugs or bioactive substances. However, further studies must be conducted.	Bandara et al. (2018)
	• Edible rapeseed protein (obtained from the oil extraction residues) films with addition of gelatin can be used as a food packages	Jang, Lim, and Song (2011)
	• Mechanical and optical properties along with water vapor barrier features of canola protein-based films were evaluated as regarding to genipin presence and plasticizer-type	Chang and Nickerson (2014)
	• Properties of canola protein-derived films, such as thermal, structural and water absorption features, strongly depend on the types of plasticizer and additives	Shi and Dumont (2014)
	• A mixture of rapeseed protein hydrolysates with chitosan can be used to produce edible composite films for food packaging	Zhang et al. (2019)
Protein-derived plastics	• Rapeseed proteins modified by succinylation can be used to produced edible films with enhanced properties	He et al. (2019)
	• Canola protein-derived plastics possesses a great potential as food biodegradable packaging, such as sachets, sacks, wraps and pouches	Zhang, Liu, and Rempel (2018)
	• Thermal and functional properties of plastics produced with improved canola proteins are higher in comparison to those with standard isolates	Manamperi et al. (2010)
	• Denaturation of used proteins can improve some properties of obtain plastics	Manamperi and Pryor (2012)
	• Canola protein-based plastic films can be used various applications, for example in compostable containers and bags, utensils, or car parts.	Bandara et al. (2018)
Superabsorbent hydrogels	• Synthesized and characterized hydrogels obtain from canola proteins show great water absorbency ability and swelling properties which respond to pH and salt content, thus might be named smart materials	Shi, Dumont, and Ly (2014)
Nanogels	• Acylated rapeseed protein isolates can be used to produce nanogels which can be used as stabilizers of Pickering emulsions in food industry	Wang et al. (2020)

dairy or meat products have been indicated (Wanasundara et al. 2016; GRAS 2016). In Tables 6 and 7 possible uses of rapeseed proteins, along with anticipated quantities are indicated. It is worth noticing that in toddler (1–3 years old) food products intended amount of rapeseed proteins use is lower than for adult group and stand at 5% in beverages, 5% in prepared food, 4% in dairy products and 3% in bakery products, versus 5% in beverages, dairy and bakery products and 10% in prepared food for adults (GRAS 2016).

Non-food and non-feed applications

Apart from having nutritional and functional properties, canola protein possesses documented bioactive features due partially to embedded bioactive peptide sequences. These peptides may be released by controlled and careful hydrolysis, which can lead to the products of potential health and pharmaceutical benefits. In previous research, bioactivity of canola proteins, including antioxidative, antithrombotic, antiemetic, anorectic, antihypertensive, antiviral, antifungal properties have been proven (Aachary and Thiyam 2012; Wanasundara et al. 2016). In Table 8 some studies on bioactivity of canola proteins and peptides are summarized.

Nowadays, a growing development of bioproducts market is observed. Canola proteins products, including plastics, adhesives and biocomposites fit into this trend. However, proteins have to be isolated and subjected to modification in an appropriate way in order to reach required features which would allow them to be used in specific applications (Manamperi et al. 2015; Manamperi et al. 2010). Sánchez-Vioque et al. (2001) acylated protein hydrolysates using C₆–C₁₆ acyl chlorides to develop products with improved foaming features. The study showed that C₁₀-acylated and C₁₆-acylated hydrolysates created more stable foams in comparison to those modified with longer and shorter aliphatic chains. Stone et al. (2013) investigated electrostatic complexes between (κ -, ι -, and λ -type) carrageenan with canola protein isolate as referred to pH value and mixing ratio of polymer's weight. The study demonstrated that solubility along with foaming and emulsifying properties of obtained complex were lower than protein isolate alone. Stone, Teymurova, and Nickerson (2014) studied functional abilities of electrostatic complexes of canola proteins isolates and gum Arabic, as referred to mixing ratio. The work showed that solubility of these materials depends on pH value. The deterioration of emulsifying and foaming capabilities for obtained complex was not observed, except for

reduced foam capacity. Also Pirestani et al. (2017) obtained conjugate of canola protein isolate and gum Arabic. The production of the complex was taking place in aqueous media by means of Maillard reaction. Obtained complex presented significantly higher solubility at isoelectric point, as compared to canola protein isolate. Further studies on canola protein isolate and gum Arabic complex obtained via Maillard reaction in terms of structural features were performed by Pirestani et al. (2018). The research demonstrated changes in tertiary structure and intensity of fluorescence. Some of possible non-food and non-feed usages of canola proteins are presented in Table 9.

Conclusions

Considering current trends in the global food markets, extending sources of supply of plant-based proteins appears to be of utmost importance. Rapeseed, which is now the second to soy oilseed crop in the world, appears to be a promising alternative. Rapeseed storage proteins: cruciferin and napin are both attributed with well-balanced amino acid composition and are perfectly suited to be used as ingredients in food and to meet human requirement for both essential and non-essential amino acids. To bring these proteins to the consumer plate, the major challenge has been so far to extract and isolate them from source material with retained nativity and functionality and using methods that are economically viable on a commercial scale. Until now, various methods for receiving rapeseed protein isolates have been reported, including basic extraction and acidic precipitation or membrane separation. The main issues concerning rapeseed processing are possible dark color, negative flavor, presence of anti-nutritional compounds or heat damage of protein during oil extraction. A few commercial canola protein products have been described, yet so far they have not been used as food components, but received Novel Food Ingredient Status given by EFSA. Studies on rapeseed proteins have showed that they generally exhibit high functional properties, such as solubility, emulsification, foam formation and gelation. However, some features of cruciferin and napin may vary, as they have different molecular structure. Methods used to obtain rapeseed products may have significant impact on their functional properties, such as solubility. Thanks to their diverse properties, rapeseed proteins are predestined to become valuable ingredients in many food formulations. The presented review indicates that rapeseed protein isolates might be incorporated in such foods as: bakery, confectionery and dairy products, beverages, dressings, sauces, meat products, snack or flavorings. They may also be used in broad range of non-food and non-feed applications as for example: adhesives, carriers or superabsorbent hydrogels.

To conclude, reviewed literature data provide compelling evidence that rapeseed protein possesses high nutritional value and functional properties, which makes it desirable food ingredient in numerous, not only vegan and vegetarian, food products. However, further studies should be conducted to demonstrate full potential of rapeseed protein products.

Disclosure statement

No potential conflict of interest was reported by the authors.

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