How might oilseeds help meet the protein challenge?

Canola/rapeseed protein-functionality and nutrition

Janitha P.D. Wanasundara1,*, Tara C. McIntosh1, Suneru P. Perera1, Thushan S. Withana-Gamage1,2 and Pranabendu Mitra1,3

1 Agriculture and Agri-Food Canada, Saskatoon Research and Development Centre Saskatoon, 107 Science Place, SK Canada, S7N 0X2
2 POS BioSciences, 118 Veterinary Road, Saskatoon, SK Canada S7N 2R4 (Current address)
3 Department of Food Science, Cornell University, 411 Tower Road, Ithaca, NY, 14853, USA (Current address)

Received 30 March 2016 – Accepted 1st June 2016

Abstract – Protein rich meal is a valuable co-product of canola/rapeseed oil extraction. Seed storage proteins that include cruciférine (11S) and napine (2S) dominate the protein complement of canola while oleosins, lipid transfer proteins and other minor proteins of non-storage nature are also found. Although oil-free canola meal contains 36–40% protein on a dry weight basis, non-protein components including fibre, polymeric phenolics, phytates and sinapine, etc. of the seed coat and cellular components make protein less suitable for food use. Separation of canola protein from non-protein components is a technical challenge but necessary to obtain full nutritional and functional potential of protein. Process conditions of raw material and protein preparation are critical of nutritional and functional value of the final protein product. The storage proteins of canola can satisfy many nutritional and functional requirements for food applications. Protein macromolecules of canola also provide functionalities required in applications beyond edible uses; there exists substantial potential as a source of plant protein and a renewable biopolymer. Available information at present is mostly based on the protein products that can be obtained as mixtures of storage protein types and other chemical constituents of the seed; therefore, full potential of canola storage proteins is yet to be revealed.

Keywords: Canola / rapeseed storage proteins / cruciferin / napin / protein digestibility / functional properties

Résumé – Protéines de canola et de colza : fonctionnalités et nutrition. Les tourteaux riches en protéines représentent un coproduit de valeur de l’extraction de l’huile de canola/colza. Dans la graine, les protéines de stockage, notamment la cruciférine (11S) et la napine (2S), dominent la fraction protéique du canola, mais des oléosines, des protéines de transfert de lipides et d’autres protéines mineures non dédiées au stockage sont également présentes. Bien que le tourteau de canola déshuillé contienne 36–40 % de protéines sur poids sec, la présence de composants non protéiques, dont les fibres, les polymères phénoliques, les phytates, la sinapine, etc. issus de l’enveloppe de la graine et des composants cellulaires rendent les protéines moins appropriées à une utilisation en alimentation humaine. Cette revue présente les connaissances actuelles en termes de valeur nutritionnelle et fonctionnelle des protéines issues des graines de canola. La séparation des protéines de canola des composants non protéiques représente un défi technique mais nécessaire pour libérer totalement le potentiel nutritionnel et fonctionnel de la protéine. Les protéines de stockage de canola peuvent satisfaire un grand nombre d’exigences nutritionnelles et fonctionnelles pour des applications alimentaires. Les macromolécules protéiques de canola offrent également des fonctionnalités requises dans les applications dépassant les seules utilisations alimentaires ; un potentiel important existe en tant que source de protéines végétales et de biopolymères renouvelables. Les informations disponibles à l’heure actuelle concernent essentiellement les produits protéiques qui peuvent être obtenus sous forme de mélanges de différents types de protéines de stockage et d’autres constituants chimiques de la graine. Tout le potentiel des protéines de stockage du canola reste donc encore à révéler.

Mots clés : Protéines de stockage / canola / colza / cruciférine / napine / digestibilité des protéines / propriétés fonctionnelles

* Correspondence: janitha.wanasundara@agr.gc.ca

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
1 Introduction

During the last 35 years, the world production of canola/rapeseed (here after referred as canola) has reached 6 times the production volume in 1980 (Fig. 1). The term canola (Canada-oil-low-acid) is defined for quality improved rapeseed genetic material consisted of <2% erucic acid containing oil and meal residue (air dried, oil-free, and 8.5% moisture containing solid) with <30 µmol of glucosinolates of any one or mixture of 3-butenyl, 4-pentenyl, 2-hydroxy-3 butenyl, and 2-hydroxyl-4-pentenyl/g. Improved germplasm of Brassica napus and Brassica rapa falls under canola. During last decade, improved Brassica juncea germplasm to fit into these criteria has been developed as canola-quality juncea. The term rapeseed or double low rapeseed is used in Europe while canola is the preferred term in Canada and Australia. However, compared to the other two, B. napus canola is mostly cultivated around the world and also the mostly researched. Canola is primarily grown for its healthy seed oil for food use however finds many applications including biofuels, cosmetics and other industrial products. The remaining seed components after oil extraction are primarily used in animal feed because of its protein, residual oil and fibre. Considering the global production in the past decade, on average, the canola crop has annually generated 10–14 million metric tons of plant protein in parallel with 20–30 million metric tons of oil (Fig. 1). Primarily, the animal feed industry is benefitted by the nutritional advantages of canola protein, especially in dairy cow rations, contributing to milk protein production. Moreover, the de-oiled canola meal is a competitive protein source that satisfies nutritional requirements of broilers, laying hens, equine and cultured fish (CCC, 2015). Canola is the second largely cultivated oilseed crop of the world after soy (OECD-FAO, 2015).

World protein demand is rising in parallel with the growing population. According to FAO/UN forecast, in 2050, the global food demand, particularly for animal protein (meat and dairy) will be twice the demand in 2013 (FAO, 2013). When global food security is considered, protein will become the limiting macronutrient and the world population will require sufficient quantities of protein with adequate quality. The growing economies with high population density are expected to demand more protein, both from animal and plant sources. In addition to the growing world population, the global concerns of environmental changes including rising greenhouse gas emission and ocean temperature, elevation of population suffering from non-communicable diseases putting pressure on public health care systems require mitigation strategies that need re-evaluating our food supply to maintain health and sustainability. Several non-communicable diseases that prevail in modern economies show the need to have a diet balanced in plant and animal sources, with the emphasis on incorporating more plant foods (Boland, 2013).

With the growing demand for protein, as a co-product of oil extraction, canola is well positioned to be a viable source of plant protein because of the volume of production and the nutritional and functional qualities of the protein. Assessment of essential amino acid profile and protein utilization efficiency in human subjects show that canola can be ranked as a high quality protein, comparable with milk and egg proteins (Bos et al., 2007; Feddermann et al., 2013). Canola protein is ranked above several plant proteins in protein quality indices and contributes more sulphur-containing amino acids and lysine than pulses, and cereals, respectively. It is a known fact that allergenic 2S proteins are part of canola seed protein complement however unavailability of long-term consumption studies and quantitative data on allergenic protein levels of canola limit providing conclusions on nutritional comparability.

The reviews published on canola protein during the last 5 years by different research groups (Aachary and Thiyam, 2012; Aider and Barbana, 2011; Alashi et al., 2013; Tan et al., 2011; Von Der Haar et al., 2014; Wanasundara, 2011) point out the significance and the value of this source as a food protein and also to generate high-value products based on protein. Environmental, demographic and economic issues we experience today highlight the advantages of direct use of plant proteins in human diet rather than converting them into animal proteins, and it is becoming a global trend as well as a need. In this context, canola has several advantages; abundance, nutritional compatibility, functional suitability, etc. This review focuses on the canola seed protein fraction for its nutritional and functional value as a plant protein source for food use. Potential uses beyond food are also discussed.

2 Proteins of canola seed

Whole canola seed or de-oiled seed (meal) is rarely or not used as a source of food protein. During 2000 to 2015, the protein content of canola seeds produced in Canada varied from 19.6–23.5% (8.5 moisture basis) while in defatted meal 37.0–41.4% (12% moisture) has been reported (CGC, 2016). Several non-protein chemical constituents of the seed are in association with protein and alter nutritional value and functional properties hindering full use of canola protein. Although the technologies developed for other oil-rich seeds such as soybean can be directly applied to separate and recover canola protein, alternative technologies and conditions are needed due to the differences in seed chemistry and protein composition.
Recoverable proteins of canola seed and meal are mostly of storage nature. Besides that some of the structural proteins such as oil body (OB) proteins, lipid transfer proteins (LTP) are also found.

2.1 Storage proteins

The storage proteins are localized in the membrane-bound protein bodies or protein storage vacuoles (PSV) that have morphologically distinct regions in the cell. According to Jiang et al. (2001) and Hu et al. (2013), three regions, the matrix, crystallloid, and globoid can be identified in canola PSV and the storage proteins are primarily in the matrix and crystallloid regions while phytate crystals are found in the globoid regions. The 11S (or 12S) globulin (legumine type), cruciferin (300–350 kDa) and 2S (or 1.7S) albumin, napin (12–16 kDa) (Crouch and Sussex, 1981; Lönnherald and Janson, 1972) are predominant storage proteins found in B. napus. Expression of cruciferin and napin in Brassicaceae species is regulated by multiple genes. A study on European cultivars support that cruciferin and napin are present in the ratio of 0.6 to 2.0 with substantial genotype variation (Malabat et al., 2003).

2.1.1 Cruciferin

Cruciferin, the predominant 11S protein in the Brassicaceae family, is a protein of cupin superfamly. Structural organization of cruciferin up to quaternary level has been described (Adachi et al., 2003; Dalgalarrondo et al., 1986; Plitz et al., 1983; Tandang-Silvas et al., 2010). The mature cruciferin contains six subunits or protomers that assemble as two trimer units in which each protomer is comprised of two polypeptide; α-(~40 kDa, 254 to 296 amino acids and β- (~20 kDa, 189 to 191 amino acids) chain linked via a disulfide bond (Adachi et al., 2003; Dalgalarrondo et al., 1986; Tandang-Silvas et al., 2010). Canola cruciferin subunits have been identified as CRU1, CRU2, CRU3, CRU4 and CRUA (Sjodahl et al., 1991; Wanasundara, 2011). In the cruciferin hexamer assembly, the inter-chain disulfide bonds between α- and β-chains play a key role (Jung et al., 1997). In the formation of hexamer the inter-chain disulfide bonds containing or IE-face1, of the two trimers pile up together via IE face-to-face. The bonds associated with assembling two trimers together are predominantly non-covalent bonds such as hydrophobic, electrostatic, hydrogen, van der Walls and hydrogen bonded salt bridges (Adachi et al., 2003).

1 According to Adachi and group (2001 and 2003), polypeptide chains of the 11S globulin protomers arrange in such a way to have the inter-chain disulfide bond less buried and located on the interface between the protomers. The intra-chain disulfide bond is more buried and located near the 3-fold axis of the trimer. In the trimer assembly, the two faces perpendicular to the 3-fold axis of trimer are referred to as the inter-chain disulfide bond containing (IE) face and the intra-chain disulfide bond containing (IA) face. The hexamer is formed by interactions between same faces (IE faces) of the trimer, mostly via hydrophobic interactions.

2.1.2 Napin

Napin, is a 2S (1.7S) protein of prolamin superfamily and exists as the next abundant storage protein of B. napus. The mature napin structure comprises of a small (short, 4 kDa) and a large (long, 9 kDa) polypeptide chain linked together by two inter-chain disulfide bonds (Shewry et al., 1995). In addition, the large chain possesses two intra-chain disulfide bonds between cysteine residues (Rico et al., 1996), making four disulfide bridges stabilizing the napin molecule. In canola, six different napin isoforms, namely Napin-1, Napin-2, Napin-3, Napin-1A, Napin-B and Nap1 have been reported in UniProtKB (http://www.uniprot.org/).

2.2 Oil body proteins

Oil body proteins (OBP) assist in stabilizing oleosomes or oil bodies (OB), which are subcellular organelles that store oils of canola seed. These proteins exhibit long hydrophobic domain that can associate with lipid phase of the droplet and a hydrophilic domain that reside on the OB surface. In B. napus, oleosins are the dominant OBP followed by steroleosins and caleosins (Jolivet et al., 2009; Tzen, 2012). Oleosins are lower in molecular mass (18–25 kDa) (Jolivet et al., 2009; Tzen et al., 1993) than caleosins (27 kDa) or steroleosins (39 or 41 kDa) (Jiang et al., 2008). Oleosins have characteristic triblock structure with two terminal amphipathic regions and a central hydrophobic region with a proline knot that is highly conserved (Hsieh and Huang, 2004; Jolivet et al., 2009). Caleosin is known to possess the ability to bind with calcium ions within the seed. Similar to oleosins, caleosins also play an important role in stabilizing OB (Tzen, 2012).

3 Nutritional value of canola proteins

Details of canola as a protein source in human diet are rare to find. Compared to the mustard counterparts of the Brassicaceae family, e.g., Brassica juncea (oriental/brown mustard), Brassica carinata (Ethiopian mustard), Brassica nigra (black mustard) and Sinapis alba (yellow or white mustard), B. napus is not used in food preparations as condiments, flavorants or preservatives. Oil-free canola meal is also not used in food. When compared with the mustard relatives, the types and levels of polymeric phenolics of the seed coat, and phenolic acids (free and esterified) and glucosinolates (α-liphatic- and indole-, total <30 μmol/g meal) of the cotyledon and embryo cells may contribute to the reported bitter and astringent taste that is not favourable to human palate. A list of foods that may use canola meal and meal protein hydrolysates is available in the dossier compiled for canola protein products (GRAS, 2010) however, these do not suggest extensive use of the protein fraction. Recent development of protein products and ingredients enabled the generation of valuable information on nutritional value of canola protein in human food.

3.1 Canola protein products

Obtaining protein rich products eliminates unwanted non-protein components of canola seed and allows better use...
of proteins. Protein product preparation from canola meal dates back to the time canola was adopted as an oilseed crop. Canola protein concentrates can be obtained by removing seed coat (reduces fibre fraction), alcohol solubles (reduce sugars, glucosinolates and some phenolics) that enrich protein content up to ~70% (Wanasundara, 2011). Preparation of protein isolates (>90% protein) that target protein in highly pure form eliminates most of the unwanted non-protein components. Depending on the method of protein extraction employed, the final product could vary in terms of the protein content, type, and extent of interaction with non-protein components. Alkali extraction and acid precipitation, protein micellation method (PMM), low pH extraction combined with membrane separation have been described for canola protein isolate preparation (Tan et al., 2011; Wanasundara, 2011). Among available information, food-grade canola protein products are described under commercial names Puratein® (precipitated micelle protein of near neutral pH protein extracted with salt, >90% protein, 11S/7S protein mainly) and Supertex® (protein remained soluble after micelle formation; Burcon Nutrascience, >90% protein, 2S protein mainly), and Isolex® (protein extracted at near neutral pH and recovered under mild conditions; TeuTexx Proteins, 60–65% globulin, remaining content albumin and other protein; EFSA, 2013).

3.2 Amino acid composition and protein quality

Canola protein provides all the nutritionally essential amino acids with a balanced amino acid profile (Tab. 1). The level of essential amino acids in canola protein and products is >400 mg/g protein (Tab. 1). The sulphur-containing amino acids (S-AA) are in the range of 3.0–4.0% or 40–49 mg/g protein, which is closer to the reference protein pattern established by FAO/UNU/WHO requirements for humans and place canola as a richer S-AA source than legume sources (Bos, 2007). Klockeman et al. (1997) identified lysine as the first limiting amino acid in canola protein and it is also the most temperature sensitive amino acid that participates in several chemical reactions including Maillard reaction (Newkirk et al., 2003). When the amino acid composition of whole canola seed or meal is compared with the protein products (Tab. 1), influence of protein composition (types) of the final product can be observed.

The protein digestibility corrected amino acid score (PDCAASS)2 for canola protein varies depending on the protein product used for assessment and also with the assessment model involving rats or weaning piglets. Rutherfurd et al. (2015) points out that the true ileal amino acid digestibility values of several plant foods (cooked forms of pea, kidney beans, rice, and rolled oats, breakfast cereals and roasted peanut), plant protein products (soy protein isolates and concentrate, rice protein concentrate, and pea protein isolate), and animal protein products (milk protein concentrate, whey protein isolate, and concentrate) obtained from rat models are comparable with the values for adult human. Therefore animal model evaluations may provide a reasonable estimation of amino acid nutrition of canola protein which is not in our regular diet.

3.3 Digestibility and amino acid nutrition

Digestibility of protein depends on the enzyme accessibility. The molecular structure as well as the other components associated with protein may affect enzyme accessibility and activity. Since early studies, glucosinolates and their breakdown products that are isothiocyanates (ITC) such as 5-vinylloxazolidine-2-thione (VOT, goitrin), butenyl-ITC, and pentenyl-ITC, and phenylethyl-ITC in addition to phytates and phenolics were considered responsible for the adverse effects observed in test animals such as reduced growth and thyroid enlargement associated with feeding rapeseed meal. These compounds may have direct effect on reducing proteolytic enzyme activity as well as bind proteins making them unavailable for enzyme-catalysed hydrolysis to peptides and amino acids. Evaluation of highly pure protein products such as isolates can eliminate the interference of non-protein components to great extent.

Early studies of Savoie et al. (1988) suggested that canola protein concentrate (52%, %N × 6.25) exhibits lower in vitro digestibility values (83%) than casein (97%) which may be attributed to the structural rigidity of canola proteins that resists acid-induced (optimum activity of pepsin is pH 1.3–2.0) denaturation and unfolding. Evaluation of rapeseed protein products in rats (Delisle et al., 1983), pigs (Grala et al., 1998) and humans (Bos et al., 2007) indicated that the canola proteins exhibit relatively poor digestibility in vivo and hydrolysate resistant protein fragments exist in the digested products. Protein efficiency ratio (PER)3 of flour, 2S and 125% of rapeseed was reported as 2.64, 2.49 and 2.12, respectively while casein under identical test conditions reported 3.23 (Delisle et al., 1983). The current FAO recommendation is to replace PDCASS with the digestive indispensable amino acid score (DIAAS)4 which uses ileal digestibility rather than fecal digestibility.

In a comparative study of different protein sources on post-prandial regional N utilization by rats, Boutry et al. (2011) showed that canola protein exhibit greater retention of N in visceral organs (small intestinal mucosa, liver and kidneys) than milk proteins which particularly enriched skin tissues. Higher content of threonine, one of the EAA that is required for mucin synthesis, and the high S-AA level of canola protein isolate may be related in promoting the retention of dietary N in visceral tissues. Canola protein isolate gave true digestibility value of 95% which was similar to milk protein in this study and it was a somewhat different observation than the low digestibility of canola protein reported in

---

2 PDCASS is a score based on the ratio of the amount of the first limiting dietary indispensable amino acid in the protein source to the amino acid requirement of the 1–2 year old child corrected for protein digestibility based on true fecal N digestibility and using the growing rat as a model for the adult human. Scores that are >1 are rounded or truncated (FAO/WHO, 1991).

3 PER is the ratio of body weight gain by a test subject to the weight of test protein consumed during a given testing period. Usually, mouse is the test subject.

4 DIAAS is a score based on ileal amino acid digestibility determined for each amino acid individually and lysine availability, using non-truncated scores (FAO, 2013).
human (Bos et al., 2007), pigs (de Lange et al., 1990; Grala et al., 1998) or chicks and cockerels (Larbrier et al., 1991). In this rat model study, canola protein isolates showed fairly close performance as milk protein for the protein nutritional parameters; similar postprandial metabolic losses of dietary N via deamination of dietary amino acids and excretion in urine, similar digestibility values, and postprandial retention of dietary N resulting in similar growth rate and body composition of rats. Similarly, Fleddermann et al. (2013) reported that canola protein isolate (soluble protein recovered from fat-free meal at pH 6.8) and canola protein hydrolysate gave 93.3% and 97.3% true nitrogen digestibility values, respectively in a rat model.

The PDCASS values reported for napin-rich Supertein™ and cruciferin-rich Puratein® (Burcon Nutrascience protein products) were 0.61 (61%) and 0.64 (64%), respectively. When calculated according to updated FAO/WHO/UNU guidelines in 2007 (WHO/FAO/UNU, 2007) values of 0.83 and 0.71 for Supertein™ and Puratein®, respectively were obtained. The limiting AA of these protein products were phenylalanine for Supertein™ and tyrosine and lysine for Puratein® (GRAS, 2010). Calculated PDCASS values for canola protein isolate and canola protein hydrolysate used in the study by Fleddermann and group (2013) were 0.86 and 1.00, respectively.

In a sub-chronic dietary toxicity assessment by 13-week rat feeding study, at 5, 10 and 20% (w/w) inclusion levels, Puratein® (cruciferin-rich) showed no negative effect on body weight gain, food consumption, blood parameters, motor activity, ophthalmic or clinical pathology observed in the animals at all feeding levels (Mejia et al., 2009a). At the 20% level of feeding, Supertein™ (napin-rich) consumed animals showed lower bodyweight (BW) gain and reduced food intake, particularly during the early weeks of feeding. Although both male and female animals showed an increase in thyroid-parathyroid weight at the 20% feeding level it was not considered as an adverse effect (Mejia et al., 2009b). For Puratein®, 10% inclusion level is recommended as the “no observed adverse effect level (NOAEL)”. This level of addition was translated into 11.24 g/kg body weight/day for male and 14.11 g/kg body weight/day for female animals (Mejia et al., 2009a). The NOAEL for Supertein™ was reported as 12.46 g/kg BW/day for males and 14.95 g/kg BW/day for females (Mejia et al., 2009b). According to this study none of these two proteins exhibited any trend to suggest genotoxic effects (GRAS, 2010).

Studies that evaluate canola protein by human feeding are limited. The study by Bos et al. (2007) assessed canola protein (36.8% globulin, 41% napin, 2.7% lipid transfer protein and total nitrogen content of 14.9%) in human subjects as a source of meal protein (27.3 g protein, 700 kcal total energy, healthy adults n = 7) and reported comparatively low (84%) real ideal digestibility values indicating low bioavailability which was compensated by the high postprandial retention of released amino acids (70.5%). Postprandial biological values reported for wheat (66%), pea (71%) or lupin (74%) were

---

**Table 1. Levels of essential, conditionally-essential and non-essential amino acids found in canola meal and protein products derived from canola.**

| Amino acid       | Canola meal, g/100 g CP | Alkali extracted acid precipitated protein isolate | Supertein™ | Puratein® | Isolex™ | 2S isolate | 11S concentrate |
|------------------|-------------------------|-----------------------------------------------|-------------|-----------|---------|------------|----------------|
| Essential        |                         |                                               |             |           |         |            |                |
| Cysteine         | 2.29                    | 0.39                                          | 4.5         | 1.6       | 2.0     | 8.1        | 1.4            |
| Histidine        | 3.39                    | 3.17                                          | 3.6         | 4.2       | 3.1     | 3.5        | 1.7            |
| Isoleucine       | 3.47                    | 5.18                                          | 3.0         | 4.4       | 4.2     | 6.0        | 6.1            |
| Leucine          | 6.19                    | 9.26                                          | 6.0         | 8.2       | 7.8     | 6.8        | 6.6            |
| Lysine           | 5.92                    | 5.62                                          | 7.4         | 4.0       | 5.5     | 3.4        | 4.6            |
| Methionine       | 1.94                    | 2.60                                          | 2.4         | 1.9       | 2.0     | 2.7        | 2.2            |
| Phenylalanine    | 4.06                    | 5.13                                          | 2.6         | 4.9       | 4.4     | 4.3        | 4.0            |
| Threonine        | 4.27                    | 5.50                                          | 3.2         | 3.7       | 4.5     | 4.5        | 4.3            |
| Tryptophan       | 1.33                    | not reported                                  | 1.4         | 2.0       | 1.5     | 1.3        | 1.2            |
| Tyrosine         | 2.50                    | 3.93                                          | 1.4         | 4.1       | 3.3     | 3.4        | 2.5            |
| Valine           | 4.97                    | 5.85                                          | 4.3         | 5.5       | 5.0     | 5.1        | 4.6            |
| Conditionally essential |             |                                               |             |           |         |            |                |
| Arginine         | 6.62                    | 7.66                                          | 5.8         | 7.2       | 7.6     | 5.4        | 5.3            |
| Glutamine+Glutamate | 18.14                | 17.27                                        | 24.6        | 19.8      | 19.8    | 14.2       | 19.8           |
| Glycine          | 4.92                    | 5.05                                          | 4.3         | 5.4       | 5.4     | 6.5        | 6.8            |
| Proline          | 5.97                    | 4.32                                          | 9.2         | 5.8       | 5.8     | 4.7        | 6.8            |
| Non-essential    |                         |                                               |             |           |         |            |                |
| Alanine          | 4.36                    | 5.14                                          | 4.0         | 4.2       | 4.5     | 5.2        | 5.3            |
| Aspartic acid+Aspartate | 7.25                | 9.41                                          | 2.6         | 9.3       | 8.8     | 11.4       | 10.5           |
| Serine           | 4.00                    | 4.74                                          | 3.3         | 4.1       | 4.9     | 5.2        | 5.5            |

a www.canolacouncil.org/media/516716/2015_canola_meal_feed_industry_guide.pdf,  b Tzeng et al., 1988,  c GRAS Notice 327, 2010,  d www.fda.gov/ucm/groups/fdagov-public/@fdagov-foods-gen/documents/document/ucm277309.pdf,  e Wanasundara and McIntosh, 2013 and Wanasundara, unpublished data.

---
proteins (each source was evaluated as their protein-enriched products obtained under mild conditions) assessed under similar test conditions were much lower than the value resulted in for canola (84%). According to this study, canola protein (protein content as %N × 6.25 was 93%, protein isolate obtained under very mild conditions) can be placed similar to egg protein that has high biological value because of the high levels of cysteine and methionine; 80% higher than the limiting value for S-AA (39.0 mg/g), and the 1:1 ratio of Met: Cys. Using the same canola protein material as the protein source, Deglaire et al. (2009) showed weaning piglet model can produce highly correlated assessment with human testing. Canola protein showed high correlation with true ileal digestibility of nitrogen \( r = 0.98 \), over \( 3 \times 2 \), \( P = 0.11 \) and amino acids \( r = 0.87 \), over \( 26 \times 2 \) data, \( P < 0.0001 \) with the data of human and weaning piglet model studies.

In the study by Fleddermann et al. (2013), both canola protein isolate and canola protein hydrolysate resulted in similar levels of incorporation of amino acids (total, essential, branched chain and non-essential) into the plasma of human subjects and the values were comparable to the soy protein isolate as dietary protein. Assessment of the same canola protein isolate and canola protein hydrolysate resulted in similar levels of cysteine and methionine; 80% higher than the limiting value for S-AA (39.0 mg/g), and the 1:1 ratio of Met: Cys.

4 Non-proteinaceous compounds associated with canola protein

Canola seed contains glucosinolates (GSL), phenolic compounds, phytates, non-starch polysaccharides that possess several advantages to the seed. Association of these compounds and their breakdown products with macromolecular protein during protein recovery processes and in the final product is considered a disadvantage due to the potential antinutrient effects, contribution to unfavorable colors and tastes. Chemistry of these components and products in canola has been reviewed in detail by Aider and Barbana (2011) and Wanasundara (2011).

Among the protein products reported for canola, cruciferin-rich Puratein® and napin-rich Supertein™ contain total intact GSL levels in the range of 1.09–2.53 and 0.39–1.02 μmol/g, respectively with no detectable levels of ITC or nitriles (GRAS, 2010) and Isolex™ (TEUTEXX Proteins, http://teutexx.com) contained GSL levels less than 0.1 μmol/g (EFSA, 2013). The canola napin isolate and cruciferin concentrate produced according to Wanasundara and McIntosh (2013) contained no intact GSLs normally associated with the seed or meal.

Phytates of canola meal are in the IP_{6} and IP_{3} form and according to Matthäus et al. (1995), commercial meal contains 15 to 21 mg/g (1.5–2.1%) and 1 to 2 mg/g (0.1–0.2%), respectively. The IP_{6} form is known to bind minerals readily, making them unavailable for intestinal absorption compared to the dephosphorylated forms (Chen, 2004; Sandberg, 2002) and also known to possess anticarcinogenic (Verghe et al., 2006) and antioxidative (Graf et al., 1987) properties. In canola protein products, the levels of phytates depend on the conditions that lead to phytate partitioning between products; Puratein® and Supertein™ were reported as 0.12–0.32% and 3.35–3.84% total phytate levels, respectively (GRAS, 2010), 1.45% phytates in the cruciferin concentrate and non detectable levels in the napin isolate prepared according to Wanasundara and McIntosh (2013), and 0.44–1.1% phytic acid level of Isolex™ (EFSA, 2013).

In canola protein product preparation, phenolic-protein complexation is difficult to avoid due to the pH, salt, and aqeous conditions involved. The level of extractable phenolics of defatted canola meal ranges from 1.59–1.84 g/100 g of defatted canola meal and 0.62–1.28 g/100 g of the seed flour (Dabrowski and Sosulski, 1984; Naczk et al., 1998). Sinapine, the choline ester of sinapic acid is the most prominent phenolic compound of canola and the contents range from 6.8–10 mg/g of seed for European cultivars (Matthäus, 1998), 6–18 mg/g of defatted meal for Canadian canola (CCC, 2016), and 16–15 mg/g of defatted meal for Australian canola (Mailer et al., 2008). Reported total phenolic acid content in Puratein® was 0.40% and Supertein™ was 0.26%, in which 93–96% was sinapic acid. Canola phenolic compounds, especially sinapinic acid, decarboxylation product 4-vinlysyringol (canolol) has strong free radical scavenging ability (Thiyam-Holländer et al., 2014; Zheng et al., 2014) but no reports are found in relation to canolol and canola protein products.

Canola seed contains free sugars consisted of glucose, fructose and sucrose up to 5%. The fibre fraction of defatted meal is consisted of cell wall fibres of the cotyledon cells, and seed coat. According to Bjergegaard et al. (1991), canola cotyledon dietary fibre (DF) has a higher negative effect on digestibility of proteins than DF isolated from seed coat, which is a consideration when meal and protein concentrates as canola protein source. Cellulose and lignins are primarily found in the insoluble dietary fibre (IDF) fraction and pectins, hemicellulose, mixed β-glucans, gums and mucilage are in the soluble dietary fibre (SDF) fraction. Ochodzki et al. (1995) have reported levels of 27.5–33.0% IDF and 3.1–5.2% SDF for spring and winter type rapeseed grown in Europe. About 2.4–6.7% and 11.8–14.0% protein were found in association with SDF and IDF, respectively as constitutive protein which was not susceptible to pepsin-pancreatin digestion (Ochodzki et al., 1995).

5 Allergenicity of canola proteins

Allergenic proteins, especially the 2S proteins of Brassicaceae seeds including canola can end up in cold-pressed canola oil (Poikonen et al., 2008; Puimalainen et al., 2006). Napin is a gastro-intestinal allergen of yellow mustard and rarely reported for inducing fatal anaphylactic reactions (Monsalve et al., 2001). Among the proteins that are capable of eliciting immunogenic response from B. napus and B. rapa seed extracts in a skin prick test, Bra n 1 (Napin BnIII, napin nIII or napin 3; P80208, 2SS3_BRANA) and Bra r 1 (Q42473, BRACM; UniProtKB/Swiss-Prot entry) were
prominent (Poikonen et al., 2008; Puumalainen et al., 2006). Presence of four S-S bonds allows tight packing and formation of a compact structure of napin. These molecular features provide special resistance to proteolytic enzyme access and thermal unfolding of napin which are the typical features of 2S albumin protein allowing them to reach the gut immune system safely as intact proteins (Mills et al., 2003). Considering the recognition of mustard (B. juncea and S. alba) and derived products as gastro-intestinal allergens of foods in EU countries and Canada, it is recommended that canola protein-containing foods need to be appropriately labelled to indicate potential allergenicity (GRAS, 2010).

6 Techno-functionalities of canola protein

Functional properties of proteins are direct manifestation of the physicochemical properties of molecules in the environment they are in and affected by the processing treatments, storage conditions and the molecules surrounding them. Protein products derived from canola contain either one type of protein or mixtures of the proteins, primarily the seed storage proteins. Investigation of polypeptide profiles of canola albumins and salt soluble globulins (Tan et al., 2011) indicates both cruciferin and napin are present in these fractions in different levels.

Functionality of proteins has an intricate relationship with its structure. According to Foezeged and Davis (2011), the functionalities of a protein that are important in food (techno-functionalities) are associated with structural transitions of the molecule such as folding in solution or at an interface. Moreover, the biological activity of a protein can be explained by structure-function relationships considering the three dimensional structure of the molecule such as certain folds, motifs and surface residues. The simple model of: Native (N) ⇔ Intermediate (I) ⇔ Denatured (D) is used in food protein functionality studies. In the reversible conversion of N structure to I state, the native tertiary structure of protein molecule is changed however the secondary structure is conserved, further unfolding of the structure without changing molecular mass or the primary structure brings the protein to D state. When canola protein products are considered, the two structurally distinct cruciferin and napin may be in N, D, or I state in different extents depending on the processing history of the starting material and protein, especially the conditions employed in seed de-oiling, de-hulling, protein extraction, recovery, concentration, drying and storage conditions. Although the tertiary structure of the cruciferin and napin have been revealed and modelled, the changes in structure in relation to D state of these proteins or the functionalities are less understood. Because of the heterogenic nature of constituent proteins, canola protein concentrates and isolates may contain cruciferin, napin and minor components; their actual contents, degree of association and level of protein structure alteration depends on the conditions employed in product preparation. Except few, most of the studies on canola protein functionality are deficient in pertinent information on processing history and product characterization (protein types and minor components), making it difficult to reach conclusive estimate of the functional potential of canola protein products.

6.1 Solubility

Solubility is a functional property highly significant in protein dispersions and has strong relationship with the functionalities of the colloidal structure development such as gelation, foaming, emulsification, and liquid (e.g., water, oil) holding. Studies on cruciferin and napin show that the solubility behaviour of these two proteins is different from each other under the conditions such as pH, temperature and salt levels (Wanasundara et al., 2012). Canola meal proteins show the least solubility between pH 3.0 and 4.0 (Wanasundara et al., 2012) although the isoelectric pH (pl, protein has zero net charge i.e. minimally or not soluble) estimated for cruciferin is pH 7.2 (Schwenke et al., 1981) and pH 11 for napin (Crouch and Sussex, 1981). Canola proteins that remain insoluble at pH 3–4 are predominantly cruciferin while napin is soluble at this pH (Wanasundara et al., 2012; Wanasundara and McIntosh, 2013). It is an indication that between pH 3 and 4, cruciferin is in complex with other chemical entities which has altered the overall charge to achieve neutrality and rendering it insoluble. Both cruciferin and napin are soluble above pH 5.5 and only napin show solubility in a wider pH range of 2 to 10. Abundance of basic amino acids in napin (Tab. 1) is exhibited as different solubility behaviour compared to cruciferin. Studies of napin of B. juncea (Jyothi et al., 2007) showed that hydrophilic nature of the molecule and the absence of hydrophobic core also influence solubility behaviour of napin while salts such as NaCl tend to stabilize napin structure by compacting. Wanasundara and McIntosh (2013) have reported that not all but a fraction of napin of B. napus and S. alba are soluble in ethanol (70–80%, v/v) and iso-propyl alcohol (30%, v/v).

6.2 Interface stabilization

Adsorption at the interface (liquid- liquid or liquid-air) and surface denaturation are necessary qualifications of a protein to perform surface activities to assist in creating protein-stabilized emulsions and foams. Adsorption of protein is mainly driven by hydrophobic interactions. Proteins are denatured at the interface to adopt a stable conformation and to minimize the interfacial free energy. Therefore, protein structure, and the extent of protein-protein and protein-solvent interactions affect adsorption and surface denaturation of protein at the interfaces (Damodaran, 2008). Considering the differences in the composition of protein types in canola protein products and the processes and inputs used for creating interfaces, the available data are specific to the study conditions tested.

6.2.1 Oil/Water (O/W) emulsions

The 11S proteins exhibit low O/W emulsifying ability because of the globular conformation maintained at the interface contributing to the low surface activity. Albumins showed high surface activity in stabilizing O/W interfaces compared to globulins (Krause and Schwenke, 2001). A high initial surface coverage in the monolayers is generated by albumins favouring more intramolecular short-range interactions.
Tan et al. (2014a) have studied albumin and globulin fractions of canola separately and showed that proteins in these fractions are capable of forming emulsions at pH 4, 7 and 9 and exhibit higher emulsifying capacity (1000–1800 ml/g) than canola protein isolate (mixture of proteins found in albumin and globulin fractions) obtained by alkali extraction and precipitation at pH 4 (500–800 ml/g) or commercial soy protein isolate (500–1500 ml/g). The emulsions formed with these proteins had average droplet size of 18–30 μm with an exceptionally large droplet size for globulin stabilized emulsions at pH 4 (80 μm). Storage stability of canola globulin and albumin stabilized emulsions at pH 4, 7 and 9 were poor compared to protein isolates. Moreover, the emulsions formed with canola (alkali extraction and pH 4 precipitation gives mixtures of cruciferin and napin) and commercial soy protein isolates at pH 7 and 9 were quite stable over a 7-day long period. Presence of phytates in canola protein isolate composed of cruciferin and napin (70% globulin, 30% albumin and ~1% phytic acid) may cause stable electrostatic protein-phytate complexes that reduce surface activity of protein molecules (Krause and Schwenke, 2001) and enhance interface stabilization. Working with somewhat pure protein, Wu and Muir (2008) and Cheung et al. (2014) showed cruciferin (>80% purity) possesses better emulsifying ability than napin (Cheung et al., 2015). Wijesundera et al. (2013) demonstrated that canola protein extracted at alkaline pH (12) and recovered by precipitation at pH 6.5 can stabilize O/W emulsions. Emulsions of tuna oil stabilized by this canola protein product showed that the unsaturated lipids can be protected against oxidation. Although these authors refer to oleosin as the major protein in the prepared protein products, SDS-PAGE profiles provided in the study clearly show presence of protein bands below 15 kDa and between 20 and 40 kDa representing S-S bonds dissociated polypeptides originating from napin and cruciferin.

### 6.2.2 Air/water foams

Air protein stabilized foam consists of dispersed air (gas) bubbles surrounded by a continuous phase of liquid in which soluble protein is at the interface (Foegeuling and Davis, 2011). The ability of a protein to form a thin film is enhanced by the unfolded structure rather than a globular compact structure (Marinova et al., 2009).

According to Nitecka et al. (1986) and Nitecka and Schwenke (1986) both 2S and 11S proteins of canola exhibit excellent foam forming and stabilizing properties; 1% (w/v) protein levels at pH 7.0 has given 440% foam expansion and 90% foam stability lasting for 10 min. Satisfactory foaming ability has been observed for the 11S canola protein obtained by protein micellation (Gruener and Ismond, 1997). The differences in the interfacial activity observed for rapeseed 11S and 2S protein in air-protein dispersions, solid phases and emulsions are related to the molecular size (Krause and Schwenke, 2001). Napin protein (93% purity) showed exceptionally high foaming ability and stability (Mitra et al., 2013) compared to cruciferin, whey protein isolate and soy protein isolate.

Protein-polysaccharide complex formation has been studied as a means of altering emulsifying and/or foaming properties of canola protein products. Using napin with high purity (>90%), Schmidt and group (2010) showed that actually pectin-protein complexes provide high foam stability in contrast to napin alone. Stone et al. (2013, 2014) utilized gum Arabic, and carrageenan (κ, λ types) to form electrostatic complexes with canola globulin fraction. Although electrostatic complexes of protein-polysaccharides are formed and exhibit reasonable functional behaviour, there hasn’t been a significant improvement in solubility, foaming properties or emulsifying properties, however these complexes can be utilized as delivery systems for small molecules such as polyunsaturated fatty acids.

### 6.3 Gel network formation

In the gel formation, protein macromolecules in sol (aqueous solutions) go through processes that increase intermolecular interactions, reach to a point that a continuous network is formed, and elasticity, a macroscopic property is developed. In foods that protein is part of, and also heat treated, the macroscopic properties of gel network structure such as moisture/fat release and the force required to cause fracture are important sensory attributes that protein contributes to heat-induced gel formation. The intermolecular interactions involved in gel formation are of covalent (disulfide bonds and iso-amide bonds) and/or non-covalent (hydrogen bonds and hydrophobic interactions, electrostatic interactions) nature and occur to various extents.

Most of the reports on canola protein gel formation are on heat-induced gelation in which thermal energy increases intermolecular interactions of protein in aqueous solutions (sols). Heat energy is capable of partial or complete unfolding of native structure (N state) of globular proteins and making buried domains of the protein available to interact inter-molecularly to form a three dimensional matrix or network. The network provides the structure and rigidity of the gel. The protein gel network is stabilized through H-bonding, hydrophobic interactions and covalent cross links such as S-S bonds (Damodaran, 2008). The thermal stability of cruciferin and napin protein structure contributes to the heat-induced gel formation property of canola protein products. Studies on canola protein gelation properties mainly describe the physical properties of the gel and their response to different factors.

The maximum gelation temperature of cruciferin and napin depends on the concentration and pH (Schwenke et al., 1998). Increase in protein concentration resulted in earlier onset of gelation of cruciferin (72 °C for 7.5% and 70 °C for 20% w/v dispersions), napin (86 °C for 7.5% and 82 °C for 20%, w/v) and also a mixture of cruciferin and napin (79.5 °C for 7.5% dispersion to 66 °C for 20% dispersion). The maximum gelation temperature of cruciferin (12.5%, w/v) at pH 7 (close pI of cruciferin) is reported as 72 °C while napin exhibited 95 °C at the same pH. The maximum gelation temperature of napin became 80 °C at pH 10 and formed a gel network that showed extreme syneresis (Schwenke et al., 1998). Napin is resistant to form a gel network between pH 4 and 8 (Folawiyoo and Apen, 1997). This may be related to the resistance to unfolding at low pH (Krzyzaniak et al., 1998; Muren et al., 1996). At pH 6, denaturation accompanied by exothermic heat effect of aggregation can be observed in napin most likely due to irreversible
denaturation and formation of hydrophobically associated aggregates. The high degree of helical secondary structure and the involvement of several S-S bonds in structure stabilization may lead to re-nature and to re-associate napin molecule upon cooling after heat-induced denaturation. This partial renaturation may result in unstable napin gels that show extreme syneresis. Below pH 4.0, napin undergoes structural modifications upon heating that lead to a significant change in surface hydrophobicity ($S_0$) indicating that its heat stability is pH dependent (Krzyżaniak et al., 1998). In contrast, the gels formed by canola globulins were stronger than napin gels and the maximum gel strength was observed around pH 7 (Krause and Schwemen, 2001). According to Yang et al. (2014), canola 2S protein in alkaline pH (15% w/v) forms gels at 120 °C. When the properties of the gels are compared, 11S protein at alkaline pH forms gel at 80 °C that possess much higher gel strength and compression strength with more particulate fractal structure than the gels formed at lower pHs. Gels of 11S protein formed at 120 °C had macro-porous structure with dense pore walls (Yang et al., 2014) which may have been facilitated from complete protein unfolding due to S-S bond dissociation.

Canola protein products that contain both cruciferin and napin form heat-induced gels, especially the gels formed at alkaline pH were more stable (Kim et al., 2016; Léger and Arntfield, 1993; Schwemen et al., 1998; Tan et al., 2014b). A range of temperatures has been observed for maximum gelation temperature of mixed canola albumin and globulin containing protein products; 69 °C for 70% globulins and 30% albumins (15% w/v protein slurry) at pH 9.0 (Schwenke et al., 1998), 88 °C for micelle isolate (Murray et al., 1985), 78.5 °C for isoelectrically precipitated isolate at pH 9 (Murray et al., 1985), and 80 °C and 81.3 °C for pH 6 and 10, respectively for 11S globulin (Léger and Arntfield, 1993). Around pH 9, globulin and napin mixture (mixed isolate) generated strong heat-set gels indicating interactions of high molecular weight cruciferin can overcome weak gel formation properties of napin. Both mixed protein isolates and individual proteins develop opaque gels (Krause and Schwemen, 2001).

Canola globulin protein gel network is established primarily by hydrophobic forces and electrostatic interactions, and gel stabilization and strengthening is attributed to disulfide bonding, electrostatic interactions and hydrogen bonding (Léger and Arntfield, 1993; Yang et al., 2014). Sodium salts promote protein molecule stability and negatively affect canola protein gel structure formation. Canola protein-hydrocolloid hybrid systems composed of up to 20% (w/w) protein (protein product had 87% protein that is composed of 3% of 2S protein and 97% of 11S or 7S) protein and 3% (w/w) κ-carrageenan resulted in gels with improved strength and structure and provided more elasticity (Uruakpa and Arntfield, 2004) but guar gum produced less elastic gels (Uruakpa and Arntfield, 2005). Structure formation and stabilization of the polysaccharide-canola protein gels were mediated by hydrophobic, noncovalent and covalent interactions (Uruakpa and Arntfield, 2006). Canola napin can induce thermal aggregation of β-casein, which can be controlled by protein concentration, pH and salt levels, and the napin aggregation was found thermoreversible (Schwartz et al., 2015).

### 6.4 Film formation

Denatured protein due to heat, acids, bases, and/or solvents can form more extended structures than the compact globular structures allowing the polypeptide chains to associate through hydrogen, ionic, hydrophobic and covalent bonding. The degree of polypeptide chain extension and the nature and sequence of amino acid residues affect chain-to-chain interaction that produces cohesive protein films while the uniform distribution of polar, hydrophobic, and/or SH groups along the polymer chain improve interactions. Improved polymer chain-to-chain interaction generates films that are stronger but less flexible and less permeable to gases, vapors and liquids. Polymers containing groups that can associate through hydrogen or ionic bonding result in films that are excellent oxygen barriers but susceptible to moisture. Protein-based edible films are used in individual packing of foods, interfaces between different layers of components of heterogeneous foods, and carriers of antimicrobials and antioxidants (Wittaya, 2012) and canola protein products may have the potential to enter in such applications.

Canola protein products (mixture of cruciferin and napin) generated films (acid denatured at pH 3 and hand casted) with much higher tensile strength, puncture strength, and elastic modulus when sorbitol was used as the plasticizer than polyethylene glycol-400 or glycerol (Chang and Nickerson, 2014). Under optimum conditions, sorbitol-canola protein film (5% protein) showed a tensile strength of 10 mPa and very low water vapor permeability. Shi and Dumont (2014) showed that water absorption of canola protein-glycerol plasticized films can be improved by adding SDS rather than fatty acids such as stearic acid. Canola napin (93% pure) can generate films with high tensile strength and low water vapor permeability by thermal denaturation combined with compression moulding at 137 °C and glycerol (up to 50%) as the plasticizer, and crosslinking with HCHO can further improve these properties (Mitra and Wanasundara, 2013).

### 6.5 Canola protein in food product applications

In order to benefit the nutritional value and functional properties of canola protein, studies on incorporation of protein products into foods as substitutes of existing protein, especially animal protein and evaluation of performance and acceptability of such products has been reported since 1970. The colour of canola protein products can range from light tan to dark brown, especially depending on the pH regime employed during processing and temperature involved in the final product drying. Alternative protein products in the market today spans over a wide range, for example, hemp protein and algal protein are not necessarily pure white or lighter in colour. It is an indication that, assurance of nutritional value, functional properties, safety and acceptable sensory (mainly taste) characteristics are the key for canola protein to stay competitive in the plant protein market. Canola protein products have been described suitable for a range of food products, including bakery products, beverages, meat binders, cheese-like products as summarized in Table 2.
Table 2. Review of edible uses reported for canola protein products.

| Bakery products                                                                 | Application                                                                                                           | Reference       |
|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------|-----------------|
| • Canola protein isolate and concentrate at 5% level in bread dough gave better results with an emulsifier. |                                                                                                                       |GRAS, 2010       |
| • Incorporation of canola protein isolate (92.2% CP) and concentrate (89.4% CP) into bread dough to replace wheat flour protein up to 18% did not cause any detrimental effect on dough and loaf quality. |                                                                                                                       |GRAS, 2010       |
| • Cruciferin-rich protein (Puratein®) isolate and napin-rich protein isolate (Supertein™) up to 2% can be incorporated into various bakery products including bread, bagels, cakes, cookies, croissant, muffins, waffles, etc. |                                                                                                                       |GRAS, 2010       |
| Dairy and egg substitutes                                                        |                                                                                                                       |GRAS, 2010       |
| • Water extracted rapeseed isolate can replace egg white protein in meringue at 3% level. Protein extracted with HCl at 3% addition level provided 10% larger specific volume in the meringue, and both protein products improved foam stability. |                                                                                                                       |GRAS, 2010       |
| • Rapeseed protein concentrate of SHMP-assisted extraction enhanced whipping properties of meringue formulations (9% dispersion and 1:1 ratio with egg white). Mixing with egg white protein (1:1.9% dispersion) helped in alleviating some of the colour and flavour problems of meringue with rapeseed concentrate. |                                                                                                                       |GRAS, 2010       |
| • Cruciferin-rich protein (Puratein®) isolate and napin-rich protein isolate (Supertein™) up to 5% in various dairy-based beverages, cream products, cheese spreads, whip cream substitutes and up to 60% in egg substitutes. |                                                                                                                       |GRAS, 2010       |
| Processed meat products                                                          |                                                                                                                       |GRAS, 2010       |
| • Rapeseed protein concentrate in wiener formulation (3.8% level) provided improved peelability of the casing, more liquid retention in beef patties (3% level of addition). |                                                                                                                       |GRAS, 2010       |
| • Canola protein isolate and concentrate to replace meat content of bologna formulation up to 3% weight, improved water holding capacity and cook yield. |                                                                                                                       |GRAS, 2010       |
| • Sausage formulation containing rapeseed protein concentrate (dehulled meal washed with isopropanol and alkali) replacing casein (2% by weight) while maintaining same meat protein and fat content scored better for aroma and taste attributes but poorer texture and colour of the cooked product than the control containing casein. |                                                                                                                       |GRAS, 2010       |
| • Cruciferin-rich protein (Puratein®) isolate and napin-rich protein isolate (Supertein™) can be incorporated up to 2% in, bologna, hot dog, ham, sausage, meat-based soups, etc. |                                                                                                                       |GRAS, 2010       |
| • Sausages with rapeseed protein isolate reduced cooking loss and replace sodium caseinate in formulations. |                                                                                                                       |GRAS, 2010       |
| Salad dressings, sauces and flavourings                                          |                                                                                                                       |GRAS, 2010       |
| • Rapeseed protein ingredients to substitute egg protein in mayonnaise-type product provided similar firmness, acid precipitated rapeseed proteins reduced firmness of mayonnaise preparation over time, and products were brown in colour. |                                                                                                                       |GRAS, 2010       |
| • Cruciferin-rich protein (Puratein®) isolate and napin-rich protein isolate (Supertein™) can be incorporated up to 2% in various salad dressing formulations including mayonnaise-type. |                                                                                                                       |GRAS, 2010       |
| • Canola meal protein hydrolysate (enzyme assisted) reacted with xylose and cysteine (optimum at pH 4, 160 °C) and generated thermal reaction products with meat flavour notes. Among the flavour active compounds, aldehydes, ketones, pyrazines, furans thiophenes, thiazoles, pyrazoles, and pyridines were identified. |                                                                                                                       |GRAS, 2010       |

7 Value of canola protein in non-food and non-feed uses

_In silico_ analysis of canola (_B. napus_) seed storage protein (cruciferin and napin) primary sequences using BioPep database (http://www.uwm.edu.pl/biochemia), Wanasundara (2011) showed that peptide sequences with proven antiinmune, antihypertensive, antithrombotic, antioxidative, anorectic, etc. are embedded in these proteins. Controlled hydrolysis of canola protein generates peptides that have potential health benefits as well as pharmaceutical value (Tab. 3) and can extend the value of canola protein beyond amino acid-based or protein functionality-based uses. As a polymer of amino acids that has multiple reactive sites and charged residues, seed proteins can be utilized in various reactions, as well as converting into molecules/polymers with diverse functionalities. Most of the studies on canola protein for such uses require obtaining protein free of fibre and other...
non-protein components. Some of these applications (Tab. 4) are quite promising in advancing economic returns for the canola crop as well as supporting the generation of renewable biopolymer and green chemicals.

### Table 3. Reported bioactivities of peptides derived from canola protein.

| Bioactivity       | Study details                                                                 | Reference                        |
|-------------------|-------------------------------------------------------------------------------|----------------------------------|
| Angiotensin I-converting enzyme (ACE) inhibiting. | • Peptides having IY, RIY, VW and VWIS sequences with ACE inhibiting activity was generated from enzyme-assisted hydrolysis of rapeseed meal. | Marczak *et al.*, 2003           |
|                   | • Sequences of VSV and FL from ACE inhibitory protein hydrolysate from canola meal. | Wu and Muir, 2008               |
| in vitro and in vivo | • Rapeseed protein hydrolysed with Alcalase generated RIY peptide has high potency as an antihypertensive component in spontaneously hypertensive rat models. | Pedroche *et al.*, 2004          |
|                   | • The potential of generating ACEI peptides from Hydrolysis of *B. napus* proteins (alkali extracted and acid precipitated) with endoprotease. | Yoshie-Stark *et al.*, 2006       |
| Antioxidative     | • Ethanol soluble peptides of rapeseed meal protein hydrolysates possessed antioxidiant activities as indicated by reducing power, hydroxyl and DPPH radical scavenging activity and ferrous-induced phosphotidyl choline oxidation inhibition, and antithrombotic activity. | Zhang *et al.*, 2008             |
| Antifungal        | • Napins recovered from commercial canola showed strong activity in suppressing growth of *Fusarium langsethiae*. | Noi *et al.*, 2012               |
| Affecting food intake | • Oral feeding of RIY peptide of canola hydrolysate exhibited anorexic effects on fasting ddY male mice and the same peptide was capable of blocking cholecystokinin-1 (CCK1) receptor antagonist lorglumide and decrease of gastric emptying rate by blocking lorglumide. | Marczak *et al.*, 2003           |
| Affecting blood sugar | • Canola protein isolates exerted preventive effects on the early onset of insulin resistance in rats fed with high saturated fat and sucrose diets. | Mariotti *et al.*, 2008          |
| Affecting cell growth | • Rapeseed protein hydrolysate with mostly 1 kDa molecules enhanced the growth of insect cell Sf9 line in serum-free media more effectively than bovine lactalbumin hydrolysate without affecting the general metabolism of the cells.  | Deparis *et al.*, 2003           |
|                   | • Rapeseed protein hydrolysates containing peptides of 0.5 to 5 kDa enhanced CHO C5 cell line growth rate. | Farges-Haddani *et al.*, 2006     |
|                   | • Canola meal hydrolysate from Alcalase showed anti-inflammatory activity of the cells. | Rivera *et al.*, 2016            |
|                   | • Alcalase-assisted hydrolysis of canola protein generated peptides capable of inhibiting human immunodeficiency virus (HIV) protease. | Yust *et al.*, 2004              |

8 Conclusions

Canola has become a stable and progressive oilseed in the global vegetable oil industry. In the changing landscape of food proteins and renewable polymers, canola has several favourable traits to become a viable plant protein source. Protein containing meal is a co-product of the food-grade canola oil extraction that can be a source of protein product preparation. Protein recovery methods for canola require special considerations because of the non-protein components of the seeds such as phenolic compounds, glucosinolates and their breakdown products, and phytates. The inherent differences of constituent proteins of canola may provide unique advantages over other plant proteins. The two predominant proteins of canola, cruciferin and napin are different in the genes that are involved in their expression, amino acid composition, structural arrangement and properties of the molecules, and abundance in the seed. Because of these inherent differences these two proteins exhibit diverse functional properties while performing differently under the conditions of food processing and preparation. Understanding the details of structure and properties of storage and structural proteins of canola is needed for optimum utilization in nutritional and functional applications.

Acknowledgements. Authors wish to thank funding support provided by Saskatchewan Canola Development Commission (SaskCanola), Agriculture Development Fund (ADF, Saskatchewan Ministry of Agriculture) and Agriculture and Agri-Food Canada (AAFC) to study and investigate canola protein for bioproduct development and allowing them to contribute in advancing the scientific knowledge on canola protein. Authors disclose no potential conflict of interest.
Table 4. Review of non-food, non-feed applications of protein fraction of canola.

| Usage                                  | Study details                                                                 | Reference                           |
|----------------------------------------|------------------------------------------------------------------------------|-------------------------------------|
| Films with water barrier properties    | Salt soluble canola protein at pH 7 was made into films with glycerol, sorbitol  | Chang and Nickerson, 2014            |
|                                        | or PEG 400 as plasticizer with or with genipin as cross linking agent by solution |                                     |
|                                        | casting, evaluated for physical properties and moisture resistance.            |                                     |
|                                        | Salt soluble canola protein isolate made into solution casted films with glycerol | Shi and Dumont, 2014                |
|                                        | as plasticizer, and SDS and stearic acid co-plasticizer. Property evaluation with |                                     |
|                                        | water absorption properties.                                                   |                                     |
| Hydrogel as superabsorbent             | Hydrogels prepared from hydrolysed canola proteins graft copolymerization of    | Shi et al., 2014                    |
|                                        | acrylic acid monomers, structural evaluation and property identification, showed |                                     |
|                                        | very high water absorbing ability with swelling and response to pH and salt.    |                                     |
| Protein-based surfactants/foams/       | • Amino groups of the canola protein hydrolysate peptides acylated with C\textsubscript{10} and C\textsubscript{12} | Sánchez-Vioque et al., 2001        |
| interface active molecules             | chains assessed for foam generation and stabilization.                        |                                     |
|                                        | • Grafting long aliphatic hydrocarbon chains and arylsulfonyl groups to lysyl residues | Gerbanowski et al., 1999; Krause, 2002 |
| Protein-based plastics                 | Canola protein isolates (alkaline extracted and acid precipitated) denatured with | Manamperi et al., 2010; Manamperi and Pryor, 2011 |
|                                        | Na dodecyl sulfate or Na dodecyl benzene sulfonate and plastic-type material prepared by thermal |                                     |
|                                        | thermal extrusion and injection moulding process with glycerol as plasticizer and |                                     |
|                                        | with co-polyester, and co-stabilizer PVP and zinc sulfate cross linker and evaluated |                                     |
|                                        | for material properties.                                                       |                                     |
|                                        | Canola napin isolates (pH 3 extracted) plasticized with glycerol and cross linked with | Mitra and Wanasundara, 2013          |
|                                        | HCHO of NaHSO\textsubscript{3}, made by compression moulding was studied for  |                                     |
|                                        | mechanical properties and water vapor barrier properties.                      |                                     |
| Protein-based adhesives                | Canola protein isolates – poly (glycidyl methacrylate) conjugated formed by free | Wang et al., 2014                   |
|                                        | radical polymerization evaluated for mechanical properties and water resistance. |                                     |
| Nanoparticles for control delivery of bioactivities | Cruciferin nanoparticles prepared from Ca-induced cold gelation, details of structure and using nanoparticles for encapsulating β-carotene for control release. | Akbari and Wu, 2016               |

References

Aachary AA, Thiyam U. 2012. A pursuit of the functional nutritional and bioactive properties of canola proteins and peptides. Crit. Rev. Food Sci. Nutr. 52: 965–979.

Adachi M, Kanamori J, Masu da T, et al. 2003. Crystal structure of soybean 1S globulin: Glycinin A3B4 homohexamer. Proc. Natl. Acad. Sci. USA 100: 7395–7400.

Aider M, Barbara C. 2011. Canola proteins: Composition, extraction, functional properties, bioactivity, applications as a food ingredient and allergenicity – a practical and critical review. Trends Food Sci. Technol. 22: 21–39.

Akbari A, Wu J. 2016. Cruciferin nanoparticles: Preparation, characterization and their potential application in delivery of bioactive compounds. Food Hydrocoll. 54: 107–118.

Alash AM, Blanchard CL, Mailer RJ, Agboola SO. 2013. Technological and bioactive functionalities of canola meal proteins and hydrosylates. Food Rev. Int. 29: 231–260.

Bjerregaard C, Eggum BO, Jensen SK, Sørensen H. 1991. Dietary fibres in olive seed rape: Physiological and antiinflammatory effects in rats of isolated idf and sdf added to a standard diet. J. Anim. Physiol. Anim. Nutr. 66: 69–79.

Boland M. 2013. Global food supply-the world’s need for protein. Available from: www.Riddet.Ac.Nz/sites/default/files/content/2013%20protein%20supply%20mike%20boland.Pdf (last consult: 2016/20/03).

Bos C, Airinei G, Mariotti F, et al. 2007. The poor digestibility of rapeseed protein is balanced by its very high metabolic utilization in humans. J. Nutr. 137: 594–600.

Boutry C, Fouillet H, Mariotti F, Blachier F, Tome D, Bos C. 2011. Rapeseed and milk protein exhibit a similar overall nutritional value but marked difference in postprandial regional nitrogen utilization in rats. Nutr. Metab. 8: 52.

CCC. 2016. Canola Meal Feeding Guide. Available from: www.canolacouncil.org/media/516716/2015_canola_meal_feed_industry_guide.pdf (last consult: 2016/29/03)

CGC. 2016. Canadian Grain Commission. Quality of western Canadian Canola. Available from https://www.grainscanada.gc.ca/canola/harvest-recette/2015/hqc15-qrc15-4-en.htm (last consult: 2016/25/05).

Chang C, Nickerson MT. 2014. Effect of plasticizer-type and genipin on the mechanical, optical, and water vapor barrier properties of canola protein isolate-based edible films. Eur. Food Res. Technol. 238: 35–46.

Chen, Q. 2004. Determination of phytic acid and inositol pentakisphosphates in foods by high-performance ion chromatography. J. Agric. Food Chem. 52: 4604–4613.

Cheung L-L, Wanasundara JPD, Nickerson MT. 2014. The effect of pH and NaCl on the emulsifying properties of a cruciferin-rich protein isolate. Food Biophys. 9: 105–113.

Cheung L, Wanasundara JPD, Nickerson MT. 2015. Effect of pH and NaCl on the emulsifying properties of a napin protein isolate. Food Biophys. 10: 30–37.

Crouch ML, Sussex IM. 1981. Development and storage-protein synthesis in brassica napus I. Embryos in vivo and in vitro. Planta 153: 64–74.

Dabrowski KJ, Sosulski FW. 1984. Composition of free and hydrolyzable phenolic acids in defatted flours of ten oilseeds. J. Agric. Food Chem. 32: 128–130.

Dalgalarrondo M, Robin J-M, Azanza J-L. 1986. Subunit composition of the globulin fraction of rapeseed (Brassica napus L.). Plant Sci. 43: 115–124.

Damodaran S. Amino acids, peptides and proteins. In: Damodaran, S, Parkin, KL, Fennema, OR (eds.). Fennema’s food chemistry CRC Press, Boca Raton, FL, USA, 2008, pp. 217–330.
de Lange CF, Souffrant WB, Sauer WC. 1990. Real ileal protein and amino acid digestibilities in feedstuffs for growing pigs as determined with the 15N-isotope dilution technique. J. Anim. Sci. 68: 409–418.

Deglaire A, Bos C, Tome D, Moughan PJ. 2009. Ileal digestibility of dietary protein in the growing pig and adult human. Brit. J. Nutr. 102: 1752–1759.

Delisle J, Amiot J, Brisson G-J, Lacroix M. 1983. Improvement of rapeseed protein nutritional value. Plant Food. Hum. Nutr. 33: 179–187.

Deparis V, Durrieu C, Schweizer M, et al. 2003. Promoting effect of rapeseed proteins and peptides on s9 insect cell growth. Cytotechnology 42: 75–85.

EFSA. 2013. Scientific opinion on the safety of “rapeseed protein isolate” as a novel food ingredient. European Food Safety Authority panel on Dietetic Products, Nutrition and Allergies. EFSA J.11(10), 3420. Available from: www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3420.pdf.

FAO. 2013. Dietary protein quality evaluation in human nutrition: Food & nutrition paper 92. Available from: www.Fao.Org/humannutrition/53978-02317b979a686a57aa4593304fc17f06.Pdf (last consult: 2016/03).

FAO/WHO. 1991. Protein quality evaluation; FAO Food & Nutrition Paper 51. Available from http://apps.who.int/iris/bitstream/10665/38133/1/9291030979_eng.pdf (Last consult: 2016/20/03).

Farges-Haddani B, Tessier B, Chenu S, et al. 2006. Peptide fractions of rapeseed hydrolysates as an alternative to animal proteins in cho cell culture media. Process Biochem. 41: 2297–2304.

Feddermann M, Fechner A, Rößler A, et al. 2013. Nutritional evaluation of rapeseed protein compared to soy protein for quality: plasma amino acids, and nitrogen balance – a randomized cross-over intervention study in humans. Clin. Nutr. 32: 519–526.

Foegeding EA, Davis JP. 2011. Food protein functionality: A comprehensive approach. Food Hydrocolloid. 25: 1853–1864.

Folawiyo YL, Apenten RKO. 1997. The effect of heat- and acid-treatment on the structure of rapeseed albumin (napin). Food Chem. 58: 237–243.

Gerbanowski A, Malabat C, Rabiller C, Guéguen J. 1999. Grafting of aliphatic and aromatic probes on rapeseed 2S and 12S proteins: Influence on their structural and physicochemical properties. J. Agric. Food Chem. 47: 5218–5226.

Graf E, Empson KL, Eaton JW. 1987. Phytic acid: a natural antioxidant. J. Biol. Chem. 262: 11647–11650.

Grala W, Verstegen MW, Jansman AJ, Huisman J, van Leeusen P. 1999. Ileal amino acid digestibilities in feedstu s for growing pigs as determined with the 15N-isotope dilution technique. J. Anim. Sci. 76: 557–568.

GRAS. 2010. GRAS Notice 327. GRAS notification for cruciferin-

Guo XF, Tian S, Small DM. 2010. Generation of meat-like flavourings from enzymatic hydrolysates of proteins from Brassica spp. Food Chem. 119: 167–172.

Hsieh K, Huang AH. 2004. Endoplasmic reticulum, oleosins, and oils in seeds and tapetum cells. Plant Physiol. 136: 3427–3434.

Hu ZY, Hua W, Zhang L, et al. 2013. Seed structure characteristics to form ultrahigh oil content in rapeseed. PloS One 8: e62099.

Jiang L, Phillips TE, Hamm CA, et al. 2001. The protein storage vacuole: A unique compound organelle. J. Cell Biol. 155: 991–1002.

Jiang P-L, Jauh G-Y, Wang C-S, Tzen JTC. 2008. A unique caloexin in oil bodies of lily pollen. Plant Cell Physiol. 49: 1390–1395.

Jolivet P, Boulard C, Bellamy A, et al. 2009. Protein composition of oil bodies from mature Brassica napus seeds. Proteomics 9: 3268–3284.

Jun R, Nam YW, Saalbach I, Munzt N, Nielsen NC. 1997. Role of the sulphydryl redox state and disulfide bonds in processing and assembly of 11s seed globulins. Plant Cell 9: 2037–2050.

Jyothi TC, Singh SA, Rao AG. 2007. Conformation of napin (Brassica juncea) in salts and monohydric alcohols: Contribution of electrostatic and hydrophobic interactions. J. Agric. Food Chem. 55: 4229–4236.

Kim JHI, Varankovich NV, Nickerson MT. 2016. The effect of ph on the gelling behaviour of canola and soy protein isolates. Food Res. Int. 81: 31–38.

Klockeman DM, Toledo R, Sims KA. 1997. Isolation and characterization of defatted canola meal protein. J. Agric. Food Chem. 45: 3867–3870.

Kodagoda LP, Nakai S, Powrie WD. 1973. Some functional properties of rapeseed protein isolates and concentrates. Can. Inst. Food Sci. Technol. J. 6: 266–269.

Krause J, Schwenke KD. 2001. Behaviour of a protein isolate from rapeseed (Brassica napus) and its main protein components – globulin and albumin – at air/solution and solid interfaces, and in emulsions. Colloids and Surfaces. B, Interointerfaces 21: 29–36.

Krause JP. 2002. Comparison of the effect of acylation and phosphorylation on surface pressure, surface potential and foaming properties of protein isolates from rapeseed (Brassica napus). Ind. Crop. Prod. 15: 221–228.

Krzyzaniak A, Burova T, Haerlé T, Barciszewski J. 1998. The structure and properties of napin-seed storage protein from rape (Brassica napus L.). FoodNahrung 42: 201–204.

Larbrrier ZM, Chagneau AM, Lessire M. 1991. Bioavailability of lysine in rapeseed and soy bean meal determined by digestibility trials in cockerel and chick growth assays. Anim. Feed Sci. Tech. 35: 237–246.

Léger LW, Arntfield SD. 1993. Thermal gelation of the 12S canola globulin. J. Am. Oil Chem. Soc. 70: 853–861.

Lönnerdal B, Janson JC. 1972. Studies on Brassica seed proteins I. The low molecular weight proteins in rapeseed. Isolation and characterization. Biochim. Biophys. Acta 278: 175–183.

Maier RJ, McFadden A, Ayton J, Redden B. 2008. Anti-nutritional components, fibre, sinapine and glucosinolate content, in australian canola (Brassica napus L.) meal. J. Am. Oil Chem. Soc. 85: 937–944.

Malabat C, Atterby H, Chaudhry Q, Renard M, Guéguen J. 2003. Genetic variability of rapeseed protein composition. Proceedings of the 11. International Rapeseed Congress. Toward Enhanced Value of Cruciferous Oilseed Crops by Optimal Production and Use of the High Quality Seed Components, pp. 205–208.

Mananperi WAR, Pryor SW. 2011. Properties of canola protein-based plastics and protein isolates modified using SDS and SDSB. J. Am. Oil Chem. Soc. 89: 541–549.

Mananperi WAR, Chang SKC, Ulven CA, Pryor SW. 2010. Plastics from an improved canola protein isolate: Preparation and properties. J. Am. Oil Chem. Soc. 87: 909–915.

Mansour EH, Dworschak E, Pollhamer Z, Gergely A, Hovari J. 2013. Seed structure characteristics to form ultrahigh oil content in rapeseed. Paper 51. Available from http://apps.who.int/iris/bitstream/10665/38133/1/9291030979_eng.pdf (Last consult: 2016/03).

Mananperi WAR, Chang SKC, Ulven CA, Pryor SW. 2010. Plastics from an improved canola protein isolate: Preparation and properties. J. Am. Oil Chem. Soc. 87: 909–915.

Mansour EH, Dworschak E, Pollhamer Z, Gergely A, Hovari J. 1999. Pumpkin and canola seed proteins and bread quality. Acta Aliment. 28: 59–70.
Marczak ED, Usui H, Fujita H, et al. 2003. New antihypertensive peptides isolated from rapeseed. *Peptides* 24: 791–798.

Marinova KG, Basheva ES, Novena B, et al. 2009. Physico-chemical factors controlling the foamyability and foam stability of milk proteins: Sodium caseinate and whey protein concentrates. *Food Hydrocolloid.* 23: 1864–1876.

Mariotti F, Herme D, Sarrat C, et al. 2008. Rapeseed protein inhibits the initiation of insulin resistance by a high saturated fat, high-sucrose diet in rats. *Brit. J. Nutr.* 100: 984–991.

Matthäus B. 1998. Effect of dehulling on the composition of antinutritive compounds in various cultivars of rapeseed. *Lipid Fett.* 100: 295–301.

Matthäus B, Lösingand R, Fiebig HJ. 1995. Determination of inositol phosphates IP3 – IP6 in rapeseed and rapeseed meal by an HPLC method, part 2: Investigations of rapeseed and rapeseed meal and comparison with other methods. *Lipid Fett.* 97: 372–374.

Mejia LA, Korgaonkar CK, Schweizer M, et al. 2009a. A 13-week sub-chronic dietary toxicity study of a cruciferin-rich canola protein isolate in rats. *Food Chem.* 47: 2645–2654.

Mejia LA, Korgaonkar CK, Schweizer M, et al. 2009b. A 13-week dietary toxicity study in rats of a napin-rich canola protein isolate. *Regul. Toxicol. Pharmacol.* 55: 394–402.

Mills ENC, Madsen C, Shewry PR, Wickers HJ. 2003. Food allergens of plant origin – their molecular and evolutionary relationships. *Trends Food Sci. Technol.* 14: 145–156.

Mitra P, Wanasundara JPD. 2013. Canola protein-based thermo plastic polymers. In Abstracts of 104th Annual meeting and expo of American Oil Chemists’ Society, Montreal, Canada.

Mitra P, McIntosh TC, Wanasundara JPD. 2013. Unique functionalities of napin protein of canola: A comparative study. In Proceedings of Canadian Society of Bioengineering Conference, Saskatoon, Canada.

Monsalve RI, Villalba M, Rodríguez, R. 2001. Allergy to mustard seeds: The importance of 2S albums as food allergens. *Internet. Symp. Food Allergens* 3: 57–69.

Muren E, Ek B, Bjork I, Rask L. 1996. Structural comparison of the precursor and the mature form of napin, the 2S storage protein in *Brassica napus.* *Eur. J. Biochem.* 242: 214–219.

Murray ED, Arnfield SD, Ismond MAH. 1985. The influence of processing parameters on food protein functionality ii. Factors affecting thermal properties as analyzed by differential scanning calorimetry. *Can. Inst. Food Sci. Technol.* J. 18: 158–162.

Naczk M, Amarowicz R, Sullivan A, Shahidi F. 1998. Current research developments on polyphenols of rapeseed/canola: A review. *Food Chem.* 62: 489–502.

Newkirk RW, Classen HL, Scott TA, Edney MJ. 2003. The digestibility and content of amino acids in toasted and non-toasted canola meals. *Can. J. Anim. Sci.* 83: 131–139.

Nitecka E, Schwenke KD. 1986. Functional properties of plant proteins. Part 8. Effect of succinylation on some functional properties of the main globulin fraction from rapeseed (*Brassica napus* L.). *Food / Nahrung.* 30: 969–974.

Nitecka E, Raab B, Schwenke KD. 1986. Chemical modification of proteins. Part 12. Effect of succinylation on some physico-chemical and functional properties of the albumin fraction from rapeseed (*Brassica napus* L.). *Food / Nahrung.* 30: 975–985.

Noi, G, Kapel R, Rondags E, Marc I. 2012. Selective extraction, structural characterisation an antifungal activity assessment of napins from an industrial rapeseed meal. *Food Chem.* 134: 2149–2155.

Ochodzki P, Rakowska M, Bjergegaard C, Sorensen H. 1995. Studies on enzymatic fractional, chemical composition and biological effects of dietary fibre in rape seed (*Brassica napus* L.). 1. Chemical composition of seeds and characteristics of soluble and insoluble dietary fibre of spring and winter type variety. *J. Anim. Feed Sci.* 4: 127–138.

OECD-FAO. 2015. Agricultural outlook 2015-2024. Available from www.Fao.Org/3/a-i4738e.Pdf (last consult: 2016/22/03).

Pedroche J, Yust MM, Megias C, et al. 2004. Utilization of rapeseed protein isolates for production of peptides with angiotensin I-converting enzyme (ACE)-inhibitory activity. *Grasas y Aceites* 55: 354–358.

Plitetz P, Damaschun G, Muller JJ, Schwenke KD. 1983. The structure of 11S globulins from sunflower and rapeseed. A small-angle x-ray scattering study. *Eur. J. Biochem.* 130: 315–320.

Poikonen S, Puimalainen TJ, Kautainen H, Palosuo T, Reumala T, Turjanmaa K. 2008. Sensitization to turnip rape and oilseed rape in children with atopic dermatitis: A case-control study. *Pediatr. Allergy Immunol.* 19: 408–411.

Puimalainen TJ, Poikonen S, Kotovuori A, et al. 2006. Napins, 2S albums, are major allergens in oilseed rape and turnip rape. *J. Allergy Clin. Immunol.* 117: 426–432.

Rico M, Bruix M, Gonzalez C, Monsalve RI, Rodriguez R. 1996. 1H NMR assignment and global fold of napin Bulb, a representative 2S albumin seed protein. *Biochemistry.* 35: 15672–15682.

Rivera D, Rommi K, Fernandes MM, Lantto R, Tzanov T. Enzyme-aided processing of rapeseed oil industry by-products into added value ingredients for food, cosmetic and pharmaceutical applications. In: White M (ed.). Rapeseed: Chemical composition, production and health benefits. NY, USA: Nova Science Publishers, 2016.

Rutherford SM, Fanning AC, Miller BJ, Moughan PJ. 2015. Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats. *J. Nutr.* 145: 372–379.

Sánchez-Vioque R, Bagler CL, Rabiller C, Guéguen J. 2001. Foaming properties of acylated rapeseed (*Brassica napus* L.) hydrolysates. *J. Colloid Interf. Sci.* 244: 386–393.

Sandberg A-S. In *in vitro and in vivo degradation of phytate.* In: Reddy NR, Sathe SK (eds.). Food phytates. Boca Raton: CRC Press, 2002.

Savoie L, Galibois I, Parent G, Charbonneau R. 1988. Sequential release of amino acids and peptides during *in vitro* digestion of casein and rapeseed proteins. *Nutr. Res.* 8: 1319–1326.

Schwartz J-M, Solé V, Guéguen J, Ropers M-H, Riaublanc A, Anton M. 2015. Partial replacement of β-casein by napin, a rapeseed protein, as ingredient for processed foods: Thermoreversible aggregation. *LWT – Food Sci. Technol.* 63: 562–568.

Schwenke KD, Raab B, Linow KJ, Pahtz W, Uhlig J. 1981. Isolation of the 12S globulin from rapeseed (*Brassica napus* L.) and characterization as a “neutral” protein. On seed proteins. Part 13. In *Particulate proteins and their applications.* (eds.) 252: 271–280.

Schwenke KD, Dahme A, Wolter T. 1998. Heat-induced gelation of rapeseed (*Brassica napus* L.) hydrolysates. *J. Colloid Interf. Sci.* 244: 386–393.

Shi W, Dumont MJ. 2014. Processing and physical properties of rapeseed protein isolate-based films. *Indus Crop. Prod.* 52: 269–277.
Shi W, Dumont MJ, Ly EB. 2014. Synthesis and properties of canola protein-based superabsorbent hydrogels. Euro. Polym. J. 54: 172–180.

Sjodahl S, Rodin J, Rask L. 1991. Characterization of the 12S globulin complex of Brassica napus. Evolutionary relationship to other 11–12S storage globulins. Eur. J. Biochem. 196: 617–621.

Stone AK, Cheung L, Chang C, Nickerson MT. 2013. Formation and functionality of soluble and insoluble electrostatic complexes within mixtures of canola protein isolate and (κ- , ϵ- and λ-type) carrageenan. Food Res. Int. 54: 195–202.

Stone AK, Teymurova A, Nickerson MT. 2014. Formation and functional attributes of canola protein isolate-gum arabic electrostatic complexes. Food Biophys. 9: 203–212.

Thompson LU, Liu RFK, Jones JD. 1982. Functional properties and food applications of rapeseed protein concentrate. J. Food Sci. 47: 1175–1180.

Tzend-Silvas MRG, Fukuda T, Fukuda C, et al. 2010. Conservation and divergence on plant seed 11S globulins on crystal structures. Biochim. Biophys. Acta (BBA) – Proteins and Proteomics. 1804: 1432–1442.

Tzend JTC, Cao Y, Laurent P, Ratnayake C, Huang AHC. 1993. Lipids, proteins, and structure of seed oil bodies from diverse species. Plant Physiol. 101: 267–276.

Tzeng YM, Diosady LL, Rubin LJ. 1988. Preparation of rapeseed protein isolates using ultrafiltration, precipitation and diafiltration. Can. Inst. Food Sci. Technol. J. 21: 419–424.

Urupaka FO, Arrnfield SD. 2004. Rheological characteristics of commercial canola protein isolate-kappa-carrageenan systems. Food Hydrocoll. 18: 419–427.

Urupaka FO, Arrnfield SD. 2005. The physico-chemical properties of commercial canola protein isolate-guar gum gels. Int. J. Food Sci. Technol. 40: 643–653.

Urupaka FO, Arrnfield SD. 2006. Surface hydrophobicity of commercial canola proteins mixed with κ-carrageenan or guar gum. Food Chem. 95: 255–263.

Verghe M, Rao DR, Chawan CB, Walker LT, Shackelford L. 2006. Anticarcinogenic effect of phytic acid (IP6): apoptosis as a possible mechanism of action. LWT 39: 1093–1098.

von Der Haar D, Müller K, Bader-Mittermaier S, Eissner P. 2014. Rapeseed proteins – production methods and possible application ranges. OCL 21: D104.

Wanasundara JPD. 2011. Proteins of Brassicaceae oilseeds and their potential as a plant protein source. Crit. Rev. Food Sci. Nutr. 51: 635–677.

Wanasundara JPD, McIntosh TC. 2013. Process of aqueous protein extraction from Brassicaceae oilseeds. US Patent 8,557963B2.

Wanasundara JPD, Abeysekara SJ, McIntosh TC, Falk KC. 2012. Solubility differences of major storage proteins of Brassicaceae oilseeds. J. Am. Oil Chem. Soc. 89: 889–891.

Wang C, Wu J, Bernard GM. 2014. Preparation and characterization of canola protein isolate-poly(glycidyl methacrylate) conjugates: A bio-based adhesive. Indus. Crop. Prod. 57: 124–131.

WHO/FAO/UNU. 2007. Protein and amino acid requirements in human nutrition. Report of a joint FAO/WHO/UNU expert consultation. WHO Technical Report Series 935. Available from: http://www.who.int/nutrition/publications/nutrientrequirements/WHO_TRS_935/en/ (Last consult:2016/20/03).

Wijesundera C, Boitoue T, Xu X, Shen Z, Watkins P, Logan A. 2013. Stabilization of fish oil-in-water emulsions with oleosin extracted from canola meal. J. Food Sci. 78: C1340–C1347.

Wittaya T. Protein-based edible films: Characteristics and improvement of properties. In: Eissa AA (ed.). Structure and function of food engineering. InTech Open Science, 2012, pp. 978–953.

Wu J, Muir AD. 2008. Comparative structural, emulsifying, and biochemical properties of 2 major canola proteins, cruciferin and napin. J. Food Sci. 73: C210–216.

Yang C, Wang Y, Vasanthan T, Chen L. 2014. Impacts of pH and heating temperature on formation mechanisms and properties of thermally induced canola protein gels. Food Hydrocoll. 40: 225–236.

Yoshie-Stark Y, Wada Y, Schott M, Wäsche A. 2006. Functional and bioactive properties of rapeseed protein concentrates and sensory analysis of food application with rapeseed protein concentrates. LWT – Food Sci. Technol. 39: 503–512.

Yust MM, Pedroche J, Megias C, et al. 2004. Rapeseed protein hydrolysates: A source of HIV protease peptide inhibitors. Food Chem. 87: 387–392.

Zhang SB, Wang Z, Xu SY. 2008. Antioxidant and anti-thrombotic activities of rapeseed pepti des. J. Am. Oil Chem. Soc. 85: 521–527.

Zheng C, Yang M, Zhou Q, Liu C-S, Huang F-H. 2014. Changes in the content of canolol and total phenolics, oxidative stability of rapeseed oil during accelerated storage. Eur. J. Lipid Sci. Technol. 116: 1675–1684.