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RESEARCH ARTICLE – DOSSIER OPEN ACCESS

HOW MIGHT OILSEEDS HELP MEET THE PROTEIN CHALLENGE?
COMMENT LES OLÉOPROTÉAGINEUX PEUVENT-ILS RÉPONDRE AU DÉFI PROTÉINES ?

Canola/rapeseed protein-functionality and nutrition

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Abstract – Protein rich meal is a valuable co-product of canola/rapeseed oil extraction. Seed storage proteins that include cruciferin (11S) and napin (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 déshuilé 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

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DOSSIER

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, 2016). 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; Fleddermann *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.

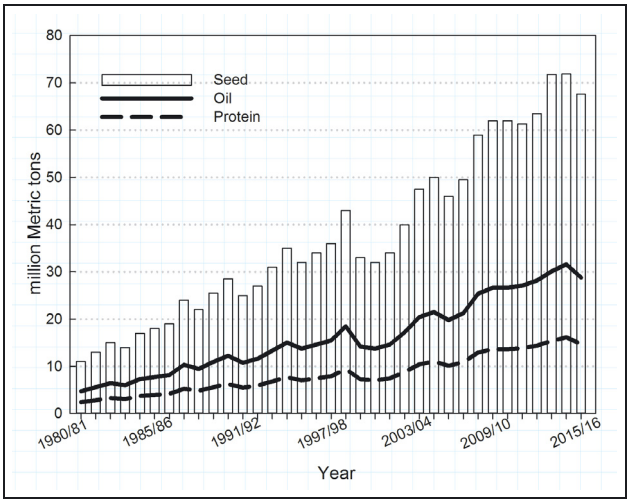

Fig. 1. Global canola production data from 1980 to 2015 and calculated production values for oil and protein based on 43% oil and 22% protein in the seed (Source: FAO, Oilseed Future).

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, crystalloid, and globoid can be identified in canola PSV and the storage proteins are primarily in the matrix and crystalloid regions while phytate crystals are found in the globoid regions. The 11S (or 12S) globulin (legumin type), cruciferin (300–350 kDa) and 2S (or 1.7S) albumin, napin (12–16 kDa) (Crouch and Sussex, 1981; Lönnerdal 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 superfamily. Structural organization of cruciferin up to quaternary level has been described (Adachi *et al.*, 2003; Dalgalarrondo *et al.*, 1986; Plietz *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-face[1]. 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 grou*p* (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-chian 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. napu*s. 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 hydropobic 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* (Ethiopean 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 (aliphatic- 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/ or7S protein mainly) and Supertein™ (protein remained soluble after micelle formation; Burcon Nutrascience, >90% protein, 2S protein mainly), and Isolexx™ (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 *et al.*, 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 (PDCASS)[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-vinyloxazolidine-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 hydrolysis resistant protein fragments exist in the digested products. Protein efficiency ratio (PER)[3] of flour, 2S and 12S fraction 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 digestible indispensable amino acid score (DIAAS)[4] which uses ileal digestibility rather than fecal digestibility.

In a comparative study of different protein sources on postprandial 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] DIASS is a score based on true ileal amino acid digestibility determined for each amino acid individually and lysine availability, using non-truncated scores (FAO, 2013).

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[a]	Canola protein products, g amino acid/100 g protein					
		Alkali extracted and acid precipitated protein isolate[b]	Supertein™, [c]	Puratein®,[c]	Isolexx™, [d]	2S isolate[e]	11S concentrate[e]
Essential							
Cysteine	2.29	0.39	4.5	1.6	2.0	8.1	1.4
Histidine	3.39	3.17	3.6	2.5	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	5.30	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.

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[5] (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 by13-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 ileal 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%)

[5] The difference is in the reference amounts of specific AA and the requirements by age groups of children 1–2 years and 3–10 years.

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 3×2, $P = 0.11$) and amino acids ($r = 0.87$, over 26×2 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 product according to European Food Safety Authority (EFSA) guide lines, it was estimated that intake of 2.2 g/kg body weight per day for "heavy" (mean + 2SD) adult consumer, 3 g/kg body weight per day for (mean) 4–6 year old group, and the 95th percentile intake of 4.73 g/kg body weight per day was acceptable (EFSA, 2013).

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 Isolexx™ (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_5 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 (Verghese *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 aqueous 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 13–15 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 sinapic acid, decarboxylation product 4-vinylsyringol (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; Puumalainen *et al.*, 2006). Napin is a gastro-intestinal allergen of yellow mustard and rarely reported for inflicting 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 Foegeding 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) $\Leftrightarrow$ Intermediate (I) $\Rightarrow$ 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 (pI, 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 thus reduces 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 precipitating 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

A protein stabilized foam consists of dispersed air (gas) bubbles surrounded by continuous phase of liquid in which soluble protein is at the interface (Foegeding 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, ι, and 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 synerisis (Schwenke *et al.*, 1998). Napin is resistant to form a gel network between pH 4 and 8 (Folawiyo and Apenten, 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 dependant (Krzyzaniak *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 Schwenke, 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; Schwenke *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 Schwenke, 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 level, 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, specially 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.

Application	Reference
Bakery products	
• Canola protein isolate and concentrate at 5% level in bread dough gave better results with an emulsifier.	Kodagoda *et al.*, 1973
• 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.	Mansour *et al.*, 1999
• 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
Beverages	
• Cruciferin-rich protein (Puratein®) isolate and napin-rich protein isolate (Supertein™) up to 10% can be incorporated into various fruit and vegetable-based juices and flavoured drinks.	GRAS, 2010
Dairy and egg substitutes	
• 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.	Kodagoda *et al.* 1973
• 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.	Thompson *et al.*, 1982
• 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	
• 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).	Thompson *et al.*, 1982
• Canola protein isolate and concentrate to replace meat content of bologna formulation up to 3% weight, improved water holding capacity and cook yield.	Mansour *et al.*, 1996
• 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.	Yoshie-Stark *et al.*, 2006
• 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.	Von Der Haar *et al.*, 2014
• Sausages with rapeseed protein isolate reduced cooking loss and replace sodium caseinate in formulations.	GRAS, 2010
Salad dressings, sauces and flavourings	
• 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.	Von Der Haar *et al.*, 2014
• 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.	Guo *et al.*, 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 antiamnestic, 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

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

Bioactivity	Study details	Reference
Angiotensin I-converting enzyme (ACE) inhibiting, *in vitro* and *in vivo*	• 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 hydolysate from canola meal.	Wu and Muir, 2008
	• 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 antioxidant 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 anti-wrinkle activity by inhibiting myleperoxidase and elastase activity.	Rivera *et al.*, 2016
Antiviral	• Alcalase-assisted hydrolysis of canola protein generated peptides capable of inhibiting human immunodeficiency virus (HIV) protease.	Yust *et al.*, 2004

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.

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.

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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 or PEG 400 as plasticizer with or with genipin as cross linking agent by solution casting, evaluated for physical properties and moisture resistance.	Chang and Nickerson, 2014
	• Salt soluble canola protein isolate made into solution casted films with glycerol as plasticizer, and SDS and stearic acid co-plasticizer. Property evaluation with water absorption properties.	Shi and Dumont, 2014
Hydrogel as superabsorbent	• Hydrogels prepared from hydrolysed canola proteins graft copolymerization of acrylic acid monomers, structural evaluation and property identification, showed very high water absorbing ability with swelling and response to pH and salt.	Shi *et al.*, 2014
Protein-based surfactants/foams/ interface active molecules	• Amino groups of the canola protein hydrolysate peptides acylated with C_{10} and C_{12} chains assessed for foam generation and stabilization.	Sánchez-Vioque *et al.*, 2001
	• Grafting long aliphatic hydrocarbon chains and arylsulfonyl groups to lysyl residues of canola cruciferin or napin, efficient way of hydrophobicizing to improve surface tension reduction in air/water interface and wettability of thin protein films.	Gerbanowski *et al.*, 1999; Krause, 2002
Protein-based plastics	• Canola protein isolates (alkali extracted and acid precipitated) denatured with Na dodecyl sulfate or Na dodecyl benzene sulfonate and plastic-type material prepared by 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.	Manamperi *et al.*, 2010; Manamperi and Pryor, 2011
	• Canola napin isolates (pH 3 extracted) plasticized with glycerol and cross linked with HCHO of $NaHSO_3$, made by compression moulding was studied for mechanical properties and water vapor barrier properties.	Mitra and Wanasundara, 2013
Protein-based adhesives	• Canola protein isolates – poly (glycidyl methacrylate) conjugated formed by free radical polymerization evaluated for mechanical properties and water resistance.	Wang *et al.*, 2014
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

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