Protein Products from Canola

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Canola or rapeseed (*Brassica napus*, *Brassica rapa* and *Brassica juncea* of canola quality) non-oil fraction contains 38-40% protein and provides the highest economic value to meal. Nutritionally, canola proteins are comparable with soybean and contain more S-amino acids than many other oilseed meals. The main research emphasis in the past has been in the use of meal protein in animal feed rather than food-grade protein products. Although substantial information on proteins of canola is available, there has been limited effort to improve protein quality or quantity through plant breeding compared to oil quality and yield improvement. The seed storage proteins are the predominant proteins in the meal protein complement. Besides that oleosins, lipid transfer proteins, and other minor proteins are found. Among the non-protein components of meal, structural carbohydrates that compose seed coat and cotyledon cells (soluble and insoluble fibre), phytates, sinapine, tannins and glucosinolates are found. The11S cruciferin and 2S napin are the abundant proteins found in canola meal. These two protein groups differ in molecular size, structural organization, physico-chemical properties and biological activities. Separation of canola protein from the non-protein components of the meal is a technical challenge. Due to the interactions and association with non-protein components, optimum nutritional and functional value of canola proteins cannot be obtained without isolation or separation. Generation of protein concentrates, isolates and unique protein fractions from canola has been described with successful demonstration of large scale production. Since protein is a co-product of canola oil extraction, the processes of protein product preparation must align with the commercial oil extraction for successful integration as an industry. Remaining non-protein components upon protein recovery such as fibre and sugars are co-product streams that can facilitate in lowering the cost of protein separation processes. In addition, compatible integration into the food systems and structures with satisfactory scientific data for regulatory approval is also essential for canola to compete as a plant protein source.

Keywords: Canola/Rapeseed storage proteins; Cruciferin; Napin; Protein concentrates; Protein isolates; Functional properties, Nutritional value

1 Introduction

Protein ingredient demand and market trends: World protein production and consumption trends and patterns have been changing in the last decade. In response to the rising demand for protein-rich food by the growing world population and the income levels of emerging economies, the agricultural and food industries are exploiting new sources while maximizing the uses of existing sources. The demand for protein-rich foods and protein ingredients includes quantity and the quality of the protein source with an added dimension of sustainability of the production chain. In the global food security considerations, protein will become the limiting macronutrient in future and the world population will require sufficient quantities of protein with adequate quality. According to the Lux Research analysis, per capita consumption rates and forecasted demographics, the demand for protein is expected to grow by 20%, reaching 569 MMT by 2025 and the market for alternative proteins beyond fish and meat is expected to have a growth of 14% by 2024 (Lux Research, 2016). With the global concerns of escalating changes in the environment and the rise of life-style related, non-communicable diseases in the populations, mitigation strategies that include re-evaluating our food supply, specifically for a diet balanced in plant and animal sources is needed with the emphasis on incorporating more plant foods. 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. In this context, canola has several advantages; abundance, nutritional compatibility, functional suitability, as the main considerations.

Opportunity for Canola/Rapeseed protein: Opportunities for canola/rapeseed in the protein market need to be assessed in both quantitative and qualitative terms. Canola seed is rich in oil (~38%), protein (~21%) and fibre (~34%) and it's production ranks as the second largest oilseed crop in the world. The quantity of protein that can be produced depends on the overall canola production, amount of that production available for protein ingredient development and overall efficiency of the protein ingredient production technologies. Considering the average level of seed protein as 21%, a production of 73.8 million tonnes of canola in 2015 has generated 15.5 million tonnes of plant protein. This hypothetical volume of canola protein is not available for human food use because a large fraction of canola meal is destined for feed use to generate animal protein (meat, milk and eggs). In the times that food protein demand is in the rise, diversion of utilization routes of plant protein sources such as canola will enable to circumvent the inefficiencies of converting plant proteins to animal proteins while providing sufficient quantities of protein to human food supply. This paper is a concise review of the status of scientific knowledge and involved technologies on canola protein and protein product generation.

2. Discussion

Nutritional value of canola protein: The quality of canola protein dictates the food use than availability of the starting material for protein production. For a food protein, the term "quality" includes both nutritional and techno-functional parameters that are critical for the product containing protein ingredient and also to the consumer. The naturally occurring non-protein compounds including polymeric phenolics of the seed coat, and phenolic acids (free and esterified) and glucosinolates and their breakdown products (aliphatic- and indole-, total <30 µmol/g meal) of the cotyledon and embryo cells considered contributing to the bitter and astringent taste of canola meal. Although the meal has ~38% protein content it is not directly included in food product development.

Nutritional assessment shows that the protein complement of canola seed provides a balanced amino acid profile with all the nutritionally essential amino acids (>400 mg/g protein) required for the human. The S-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. According to Bos *et al.* (2007) canola is a richer S-AA source than any other vegetable protein including legumes. Lysine is reported as the first limiting amino acid in canola protein by Klockeman and group (1997) and it is the most temperature sensitive amino acid that participates in several chemical reactions including Maillard reaction. The protein digestibility corrected amino acid score (PDCASS) for canola protein varies depending on the protein product used for assessment and also with the assessment model involved; rats or weaning piglets. According to the nutritional assessments in human subjects, canola ranks as a high quality protein, comparable with milk and egg proteins above most of the plant proteins in protein quality indices and in the bio-availability of amino acids (Bos *et al.*, 2007, Fleddermann *et al.*, 2013).

Cruciferin (11S globulin of legumin type, 300-350 kDa) and napin (1.7/2S albumin type, 12-16 kDa) are the two main storage proteins of canola seed and possess different amino acid compositions (Crouch & Sussex, 1981; Lönnerdal & Janson, 1972) and molecular structures. The abundance of these two proteins influences the amino acid profile of the meal and protein products. These protein are found in the membrane-bound protein bodies or protein storage vacuoles (PSV) which are morphologically distinguished in the cell (Wanasundara et al., 2016) along with phytate crystals (Jiang *et al.*, 2001; Hu *et al.*, 2013).The non-storage proteins such as oil body (OB) proteins and lipid transfer proteins (LTP) can also be recovered and included in the final protein products depending on the methods and conditions employed. Besides these the immunogenic properties of consisting proteins are also a consideration in assessing the nutritional value. Among the proteins of *B. napus* Bra n 1 (Napin BnIII, napin nIII or napin 3; P80208, 2SS3_BRANA) is identified as proteins responsible for immunogenic responses (Monslave et al., 2002; Puumalainen *et al.*, 2006; Poikonen *et al.*, 2008). Yellow/white mustard (*Sinapis alba*) and brown/oriental mustard (*B. juncea*) which share several phylogenetic and phytochemical relationships with *B. napus* are listed as allergenic sources in Canada and EU. The availability of these nutritionally essential amino acids depends on the nature of protein source, particularly, the purity (within seed matrix or in concentrated and isolated form), associated

compounds (cellulose, hemicellulose, pectin, lignin, phytates, phenolics, glucosinolate break-down products, and simple carbohydrates) and the process-induced changes all which affect digestibility and bioavailability.

Production of canola-based protein products: Natural association of canola storage protein with non-protein components of the seed are not that well investigated. Commercial oil extraction process that involves pressure, temperature and solvents such as hexanes can induce modifications to the proteins and interactions with non-protein compounds, and resulting in low protein recovery and quality. Therefore food-related nutritional and functional quality parameters are always reported on protein products obtained from canola meal under low temperature defatting. Figure 1 summarises the processes that are conceptually different in recovering protein from the meal, therefore providing products with distinct quality. The protein concentrates are produced by removing most of the residual oil and water-soluble non-protein components to have protein content not less than 65% (on a moisture-free basis) and the protein isolates are prepared by isolating proteins from other non-proteinaceous components with a protein content of at least 90% on a moisture-free weight basis (Golbitz, 2008).

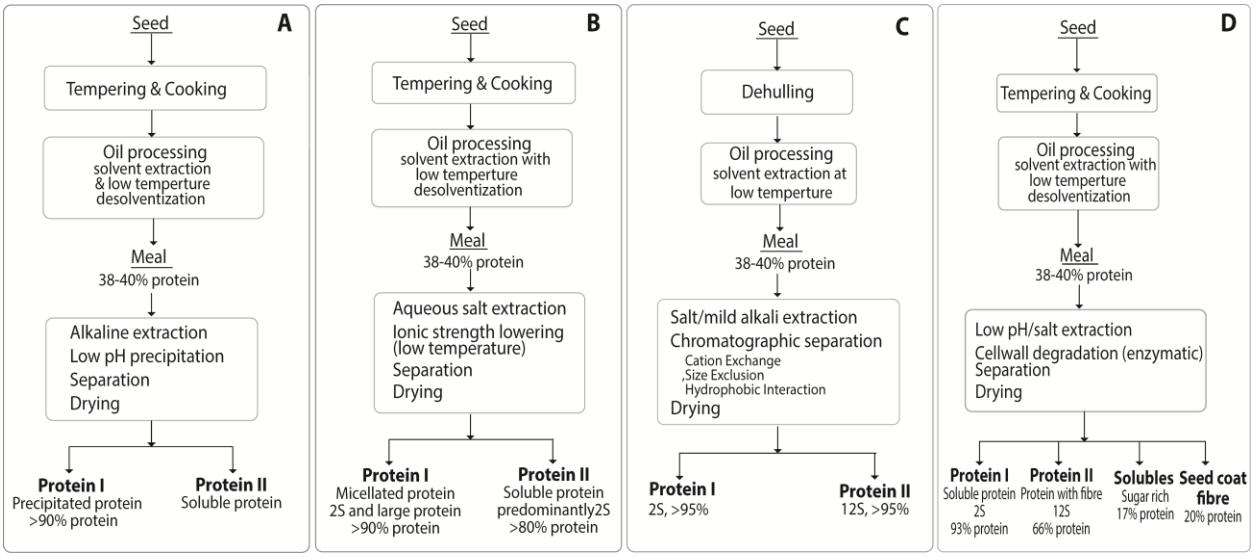

Figure 1. Distinct processing regimes for canola protein found in patent literature. A: Alkali extraction of protein and recovery at low pH (Diosady et al., 2005, Newkirk et al., 2009), B: Protein micelle formation method (Murray, 1997; 1999; Schwizer and Greene, 2005), C: Chromatographic separation (Berot et al., 2005) and D: Meal component fractionation method developed by Wanasundara & McIntosh (2013). Adapted from Wanasundara et al., 2016.

When canola protein products are considered, most of the nutritional and functional information are available for protein isolates napin-rich Supertein™ and cruciferin-rich Puratein® that was developed by Burcon Nutrascience protein products and came to near commercial stage. The PDCASS values of 0.61 (61%) and 0.64 (64%), respectively were reported for these products. When calculated according to updated FAO/WHO/UNU guidelines in 2002 (WHO/FAO/UNU, 2007; reference amounts of specific AA and the requirements by age groups of children 1-2 years and 3-10 years) values of 0.83 for Supertein™ and 0.71 for Puratein® have been reported. The limiting AA of these protein products were phenylalanine for Supertein™ and tyrosine and lysine for Puratein® (GRAS, 2010). Toxicological assessment of these protein products in 13-week rat feeding studies indicated up to 20% inclusion levels Puratein®(cruciferin-rich) showed no negative effect on body weight gain, food consumption, blood parameters, motor activity, ophthalmic or clinical pathology (Mejia *et al.*, 2009a). Feeding Supertein™ (napin-rich) at 20% level indicated lower bodyweight (BW) gain and reduced food intake, particularly during the early weeks of feeding with an increase in thyroid/parathyroid weight(both male and female animals) that was not considered as an adverse effect (Mejia *et al.*, 2009b). This study suggested that the ‘no observed adverse effect level (NOAEL)” for

Puratein® is at 20% level (11.24 g/kg body weight/day for male and 14.11g/kg body weight /day for female) and 10% inclusion level for Supertein™ (12.46 g/kg BW/day for males and 14.95 g/kg BW/day for females) (Mejia *et al.*, 2009a; 2009b). According to this study none of these two proteins exhibited any trend to suggest genotoxic effects (GRAS, 2010). 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).

Canola protein isolate prepared as 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 assessment (Fleddermann *et al*., 2013). 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. 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 (Fleddermann et al., 2013).

Total glucosinolate (GSL) levels of 1.09-2.53 and 0.39-1.02 µmol/g, respectively were reported for cruciferin-rich Puratein® and napin-rich Supertein™ products with no detectable levels of isothiocyanates or nitriles (GRAS, 2010) The protein product Isolexx™ (TEUTEXX Proteins, http://teutexx.com) which is produced from membrane filtered aqueous extracts of near neutral pH 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 GSL that are 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. 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 Isolexx™ (EFSA, 2013).

Techno-functionalities of canola protein: Functional properties of protein ingredients are the most important determinants of their use in food products. The conditions of the environment and the processing treatments applied to protein containing matrix/product alters and changes manifestation of the physicochemical properties of protein molecules therefore the functionalities they provide. Protein products derived from canola contain either one type or mixtures of seed storage proteins and/or other non-storage proteins (Tan *et al.*, 2011; Wanasundara, 2011). Comparative studies on purified napin and cruciferin show that these proteins have distinct functionalities that can be related to their differences in molecular structure and amino acid composition ((Krause and Schwenke, 2001; Wanasundara et al. 2012; Perera et al, 2016) therefore it can be expected that functionalities of canola protein ingredients depend on the consisting protein types. Besides that the type and level of non-protein polysaccharides, phenolic compounds, insoluble fibre) also play a role and modify functionalities to different extents.

Solubility of a protein is a key functionality that has strong relationship with the functionalities of the colloidal structure development by protein such as gelation, foaming, emulsification, and liquid (e.g., water, oil) holding. Since cruciferin and napin show quite distinct solubility behaviour in relation to pH, temperature and ionic strength of the aqueous dispersion (Wanasundara *et al.*, 2012; Perera et al., 2016) definitely the abundance of these proteins may dictate solubility of canola protein ingredient. Cruciferin remains insoluble at pH 3-4 while napin is soluble at this pH; both cruciferin and napin are soluble above pH 5.5 and only napin show solubility in a wider pH range of 2 to 10 (Wanasundara *et al.*, 2012, Wanasundara and McIntosh, 2013).

According to (Krause and Schwenke, 2001) the 11S proteins exhibit low O/W emulsifying ability and albumins showed high surface activity in stabilizing O/W interfaces compared to globulins. Tan et al. (2014a) showed that

proteins in canola albumin and globulin fractions are capable of forming emulsions at pH 4, 7 and 9 and exhibit higher emulsifying capacity (1000-16000 mL/g) than canola protein isolate obtained from alkali extraction and precipitation at pH 4 (500-800 mL/g) or commercial soy protein isolate (500-1500 mL/g). T According Wu and Muir (2008) and Cheung and others (2014), cruciferin (>80% purity) showed better emulsifying ability than napin (Cheung et al., 2015). In air/water interface (e.g. foams) stabilization, napin has exceptional ability compared cruciferin (Mitra *et al.*, 2013) which corroborates with the high forming ability described for Supertein™.

Cruciferin and napin exhibit different gelation behaviour and also the gelation respond to pH. Napin is resistant to form a gel network between pH 4 and 8 (Folawiyo and Apenten, 1997) and recently Perera et al. (2016) confirmed that napin cannot be coagulated by heat. Alkaline pH and 120 ºC favours gel formation of canola 2S protein (15% w/v) but with inferior qualities such as particulate gel and lower gel strength compared to the gels formed with 11S protein dispersion at same pH at 80 ºC (Yang et al., 2014).According to Krause and Schwenke (2001), at 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.

Several researchers have tried relating to bioactivities of canola protein derived peptides which can enhance the usability of canola protein. Among the reported activities, angiotensin I-converting enzyme inhibiting (ACEI) ability is more prominent (Marczak et al., 2003; Pedroche et al., 2004; Wu et al., 2008; Yoshie-Stark et al., 2006) and the active di-and tri-peptides can be generated by both napin and cruciferin. The tri peptide RIY which showed delayed gastric emptying and increasing satiety and reduction of food intake) is restricted to the primary sequence of napin (Marczak et al., 2003).

3. Conclusions

As a stable and continuously evolving oilseed, canola has a special place in global vegetable oil industry. Protien fraction has several opportunities to infiltrate into growing plant protein ingredient market while incorporating and utilizing the knowledge on seed constituents and its protein.

Acknowledgements: Saskatchewan Canola Development Commission (SaskCanola), Agriculture Development Fund (ADF, Saskatchewan Ministry of Agriculture) and Agriculture and Agri-Food Canada (AAFC) are acknowledged for funding the research on canola protein fraction.

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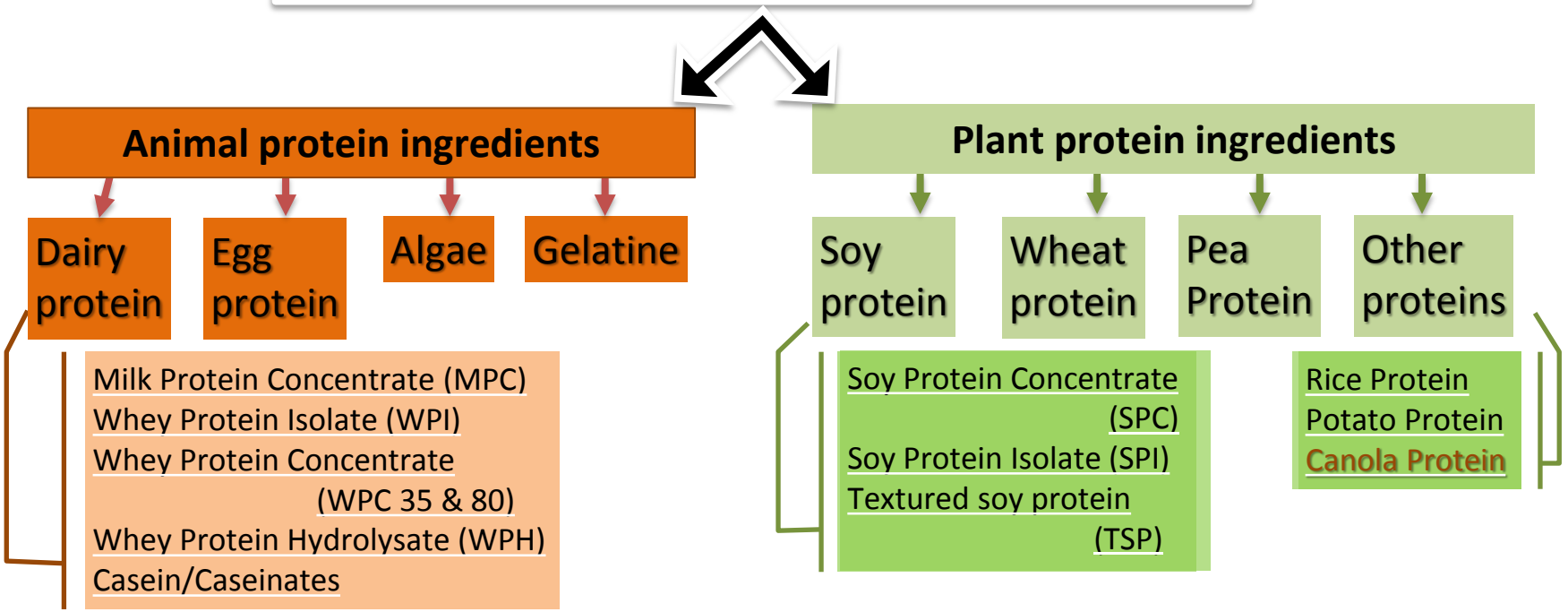
GCIRC Meeting
May 09-11, 2017

OUTLINE

- **Nature of Protein Ingredient market**
- **Why canola/rapeseed?**
- **Comparison of:**
  -basic requirements &
  -production technologies
- **Positioning Canola protein**

Global Protein Ingredient Market



Nature of the Market

- Highly fragmented
- Competition for limited number of end applications
- Identifying and addressing opportunities and threats

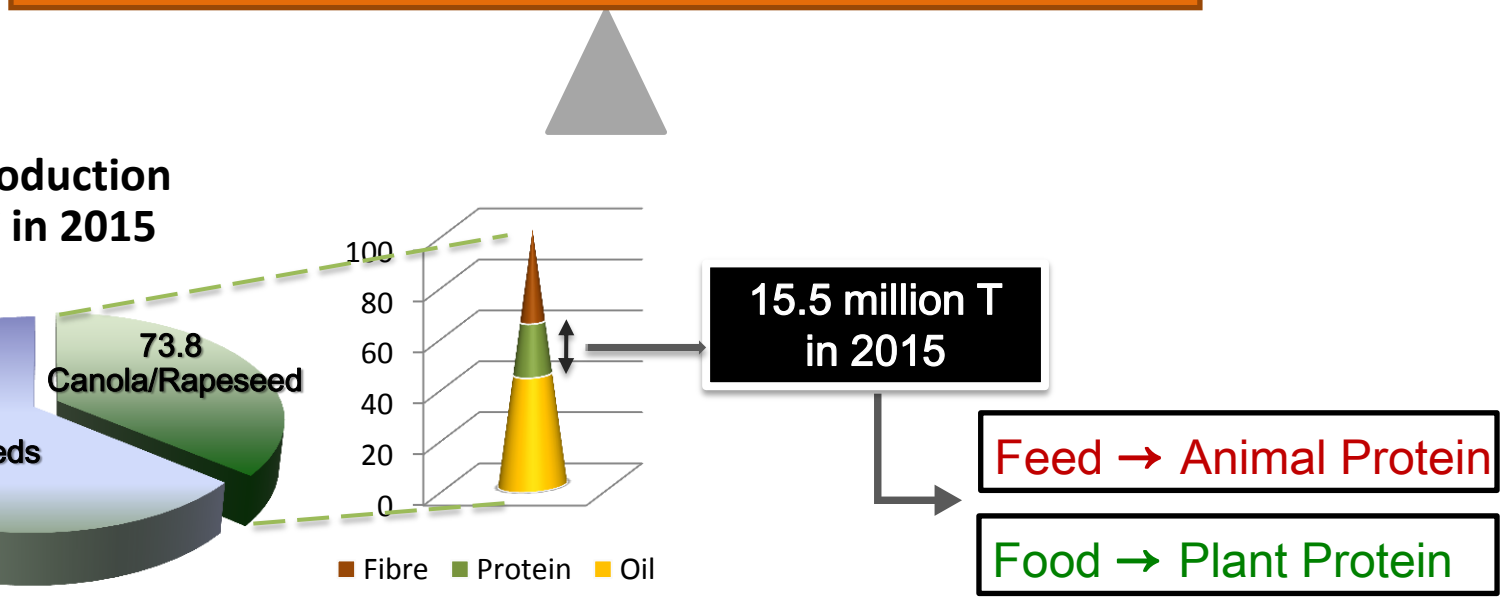
Growth factors

- Demand for improved functionality from specialized protein ingredients
- Emerging regions/economies & consumer trends create new opportunities
- Industry' drive to promote potential health benefits

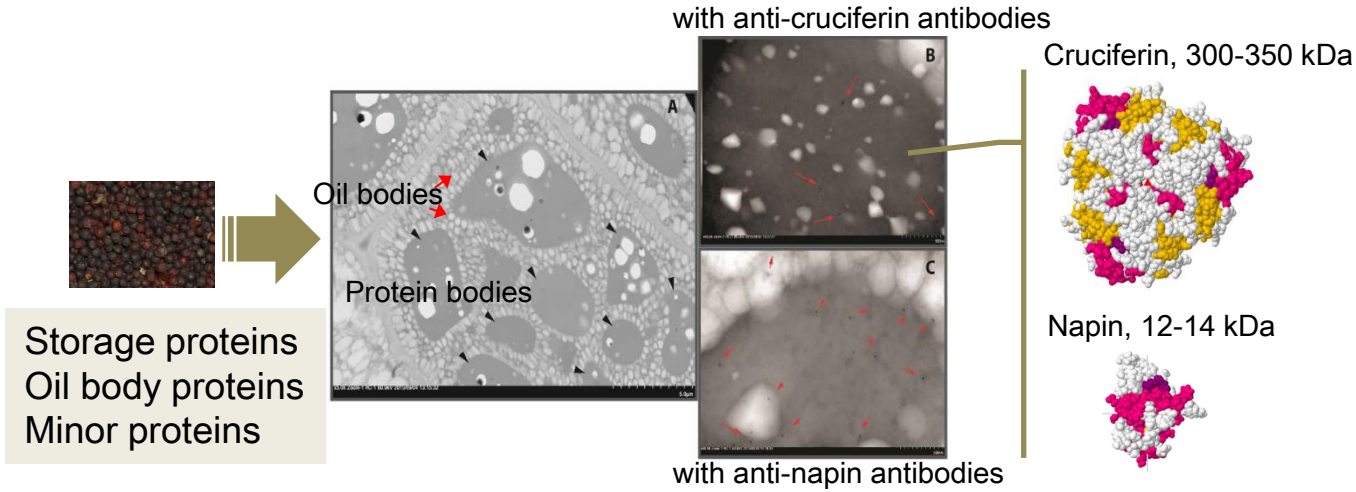
Frost & Sullivan Reports, 2015

How does Canola/Rapeseed protein weigh?

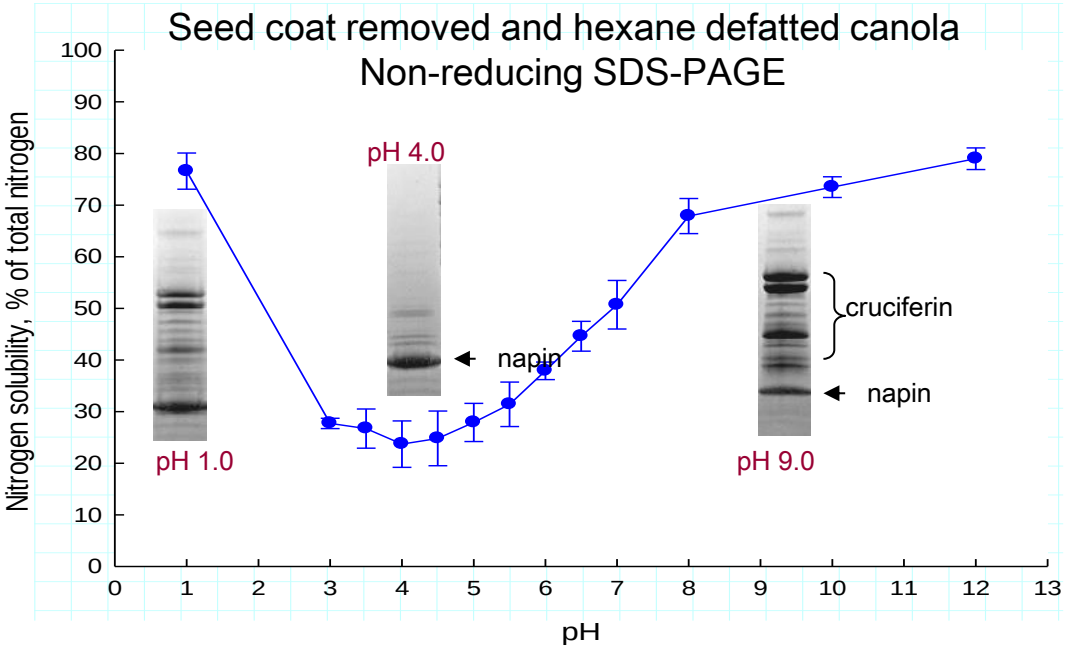
Food	Feed	Nutritional value	Functional value	Food	Feed
√	√	•Essential amino acids	•Physico-chemical		
		Inherent	Solubility	√ -?	√-?
		Processing effects	Interfacial activity	√ -?	-
√ -?	√	•Digestibility	Temp stability	√ -?	-
		Structure	Rheological	√ -?	-
		Other components	Color	√ -?	-
√-?	?	•Allergenicity/Toxicity	• Flavor & Taste	√ -?	?



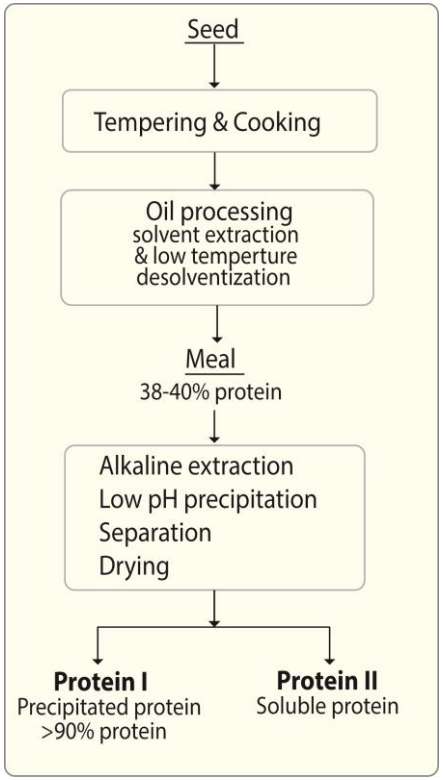
Nature of proteins in canola



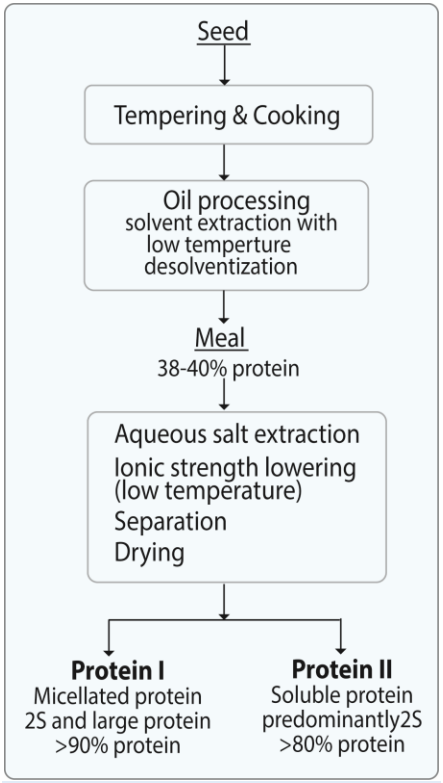
Storage proteins
Oil body proteins
Minor proteins



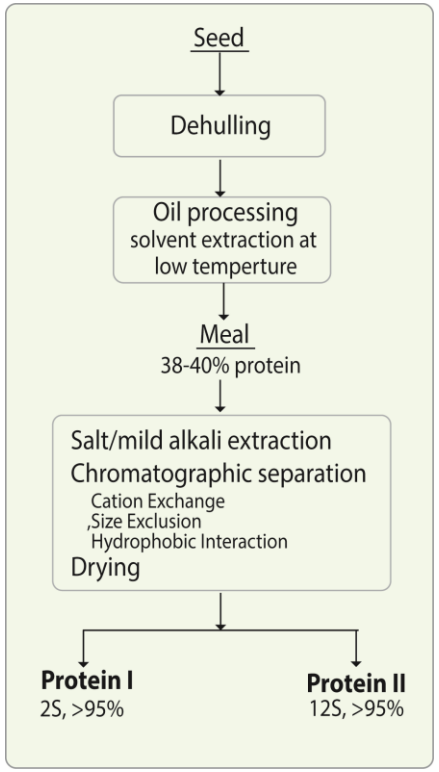
Technologies for canola protein production



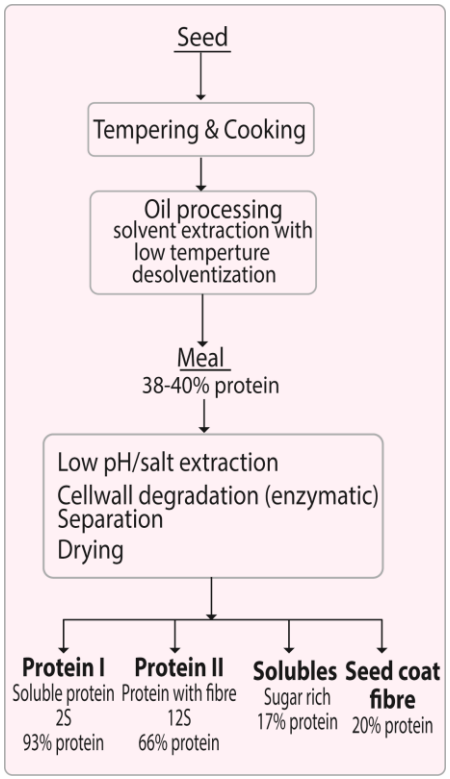
- Similar to Soy
- Diosady et al. 2005; Newkirk et al. 2009



- PMM
- Murray, 1980; 1999; Schweizer & Greene, 2005



- High purity CRU & NAP
- Berot et al., 2005



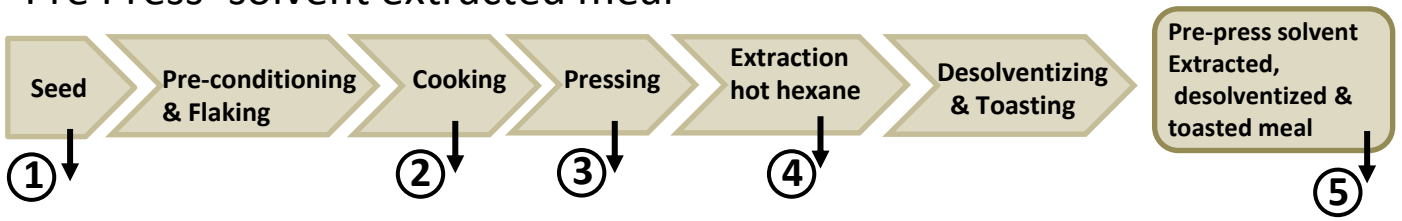
- Many products
- CRU & NAP, enriched
- AAFC 2013

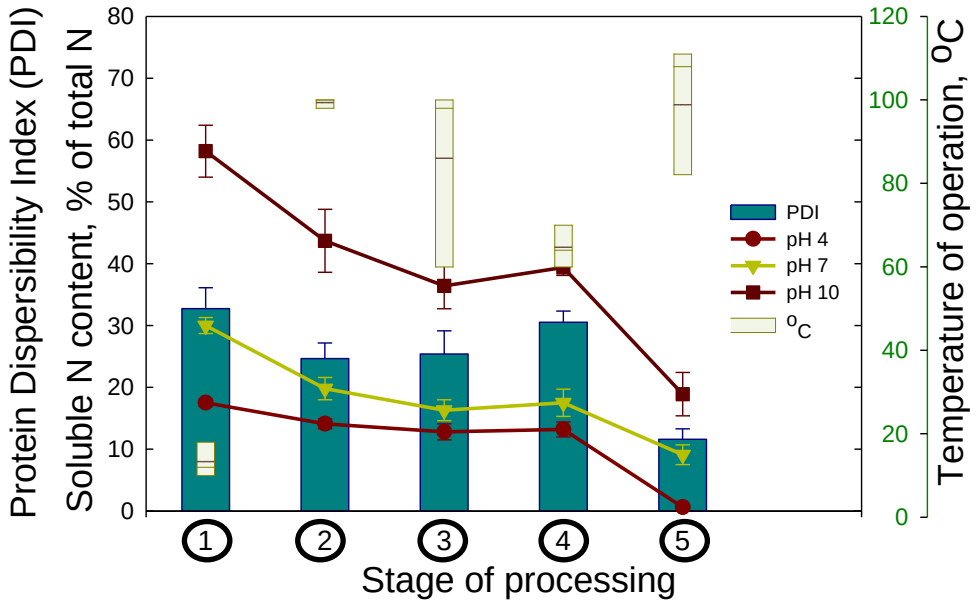
***Starting material – deoiled seed (not commercial canola meal)**

Nature of feed stock for protein production

Expeller Pressed meal

Pre Press–solvent extracted meal





Amino acid composition of canola protein & protein products

Amino acid	EAA Requirement, Adults[6]	Canola meal, g/ 100g CP[1]	Canola protein products, g amino acid/100 g protein					
			Alkali extracted & acid precipitated protein isolate[2]	Supertein™, [3]	Puratein®, [3]	Isolexx™, [4]	2S isolate, [5]	11S concentrate[5]
Essential								
Cysteine	**3.7 (+Met)**	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	**2.9**	3.47	5.18	3.0	4.4	4.2	6.0	6.1
Leucine	**4.1**	6.19	9.26	6.0	8.2	7.8	6.8	6.6
Lysine	**3.2**	5.92	5.62	7.4	4.0	5.5	3.4	4.6
Methionine	**(with Cys)**	1.94	2.60	2.4	1.9	2.0	2.7	2.2
Phenylalanine	**1.9 (+Tyr)**	4.06	5.13	2.6	4.9	4.4	4.3	4.0
Threonine	**1.9**	4.27	5.30	3.2	3.7	4.5	4.5	4.3
Tryptophan	**1.0**	1.33	not reported	1.4	2.0	1.5	1.3	1.2
Tyrosine	**(with Phe)**	2.50	3.93	1.4	4.1	3.3	3.4	2.5
Valine	**3.6**	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		1814	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

[1] www.canolacouncil.org/media/516716/2015_canola_meal_feed_industry_guide.pdf , [2]Tzeng et al., 1988, [3] GRAS Notice 327, 2010, [4] www.fda.gov/ucm/groups/fdagov-public/@fdagov-foods-gen/documents/document/ucm277309.pdf , [5]Wanasundara and McIntosh, 2013 and Wanasundara, unpublished data , FAO/WHO (2002)

Nutritional and Toxicological studies on canola protein

The Journal of Nutrition 137 (2007) 594-600
Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

The Poor Digestibility of Rapeseed Protein Is Balanced by Its Very High Metabolic Utilization in Humans[1]

Ce´cile Bos,[2]* Gheorghe Airinei,[2,3] Franxcois Mariotti,[2] Robert Benamouzig,[3] Serge Be´ rot,[4] Jacques Evrard,[5] Evelyne Fe´ nart,[6] Daniel Tome´ ,[2] and Claire Gaudichon[2]

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Clinical Nutrition 32 (2013) 519-526
Contents lists available at SciVerse ScienceDirect
Clinical Nutrition

Randomized control trials

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

Manja Fleddermann[a], Anita Fechner[a], Andrea Rößler[a], Melanie Bähr[a], Anja Pastor[b], Frank Liebert[b], Gerhard Jahreis[a],*

[a]Department of Nutritional Physiology, Institute of Nutrition, Friedrich Schiller University, Dornburger Str. 24, D-07743 Jena, Germany
[b]Department of Animal Sciences, Division Animal Nutrition Physiology, Georg August University, Goettingen, Germany

British Journal of Nutrition (2009), **102**, 1752–1759 doi:10.1017/S0007114509991267
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Ileal digestibility of dietary protein in the growing pig and adult human

Amélie Deglaire[1,2], Cécile Bos[2], Daniel Tomé[2] and Paul J. Moughan[1]*
[1]*Riddet Institute, Massey University, Private Bag 11222, Palmerston North, New Zealand*
[2]*INRA, AgroParisTech, UMR914 Nutrition Physiology and Ingestive Behaviour, CRNH-IdF, 75005 Paris, France*

Boutry *et al. Nutrition & Metabolism* 2011, **8**:52
http://www.nutritionandmetabolism.com/content/8/1/52

Nutrition&Metabolism

RESEARCH Open Access

Rapeseed and milk protein exhibit a similar overall nutritional value but marked difference in postprandial regional nitrogen utilization in rats

Claire Boutry [1,2], Hélène Fouillet[1,2*], François Mariotti[1,2], François Blachier[1,2], Daniel Tomé[1,2] and Cécile Bos[1,2]

Regulatory Toxicology and Pharmacology, Volume 55, Issue 3, 2009, Pages 394–402

A 13-week dietary toxicity study in rats of a Napin-Rich Canola Protein Isolate

Luis A. Mejia [a],*, Chandrashekhar K. Korgaonkar [b], Martin Schweizer [c], Christopher Chengelis [b],Meliton Novilla [b], Ellen Ziemer [b], Patricia S. Williamson-Hughes [a], Richard Grabiel [a], Mark Empie [a]
[a] Archer Daniels Midland Company (ADM), 1001 North Brush College Road, Decatur, IL 62521-1656, USA
[b] WIL Research Laboratories, LLC, 1407 George Road, Ashland, OH 44805-8946, USA
[c] Burcon NutraScience Corporation, 1388 Waller Avenue, Winnipeg, MB, Canada R3T 1P9

Food and Chemical Toxicology, Volume 47, Issue 10, 2009, Pages 2645–2654

A 13-week sub-chronic dietary toxicity study of a cruciferin-rich canola protein isolate in rats

Luis A. Mejia[a], ,Chandrashekhar K. Korgaonkar[b], Martin Schweizer[c], Christopher Chengelis[b], Gary Marit[b], Ellen Ziemer[b], Richard Grabiel[a], Mark Empie[a]

Amino acid digestion and availability of canola protein products

Amino acid composition, g/ 100 g protein

Egg	Adult Req.	Amino acid	Napin isolate	Cruciferin concentrate	SPC	SPI
4.0	3.7	Cysteine + Methionine	4.3 + 2.2	1.0 +1.4	0.6 + 0.7	1.3
4.3	2.9	Isoleucine	2.7	3.0	3.2	4.9
7.1	4.1	Leucine	5.3	5.2	5.1	8.2
7.0	3.2	Lysine	6.9	3.9	3.9	6.3
9.3	1.9	Phenylalanine + Tyrosine	2.6 + 1.7	3.3 + 2.7	3.5 + 2.5	5.2
1.7	1.0	Tryptophan	1.1	1.0	0.8	1.3
3.4	1.9	Threonine	3.4	3.4	2.8	3.8
5.2	3.6	Valine	3.7	3.6	3.1	5.0
		Aspartic acid & Asparagine	3.4	5.8	7.5	
		Glutamic acid & Glutamine	21.1	8.0	11.0	
		Serine	3.7	3.6	3.9	
		Glycine	4.0	3.2	2.6	
		Histidine	3.5	1.7	1.8	
		Arginine	5.4	3.8	4.6	
		Alanine	3.4	2.8	2.6	
		Proline	7.0	3.1	3.2	

Wanasundara (unpublished data)

True ileal digestibility (%) values*

Napin isolate	Cruciferin concentrate	SPC
83.6 & 84.1	76.3 & 73.1	66.5 & 80.2
78.8	81.4	78.9
77.8	82.5	80.3
74.2	75.9	75.0
80.0 & 84.2	83.8 & 79.0	81.9 & 78.2
87.5	81.2	78.3
70.1	73.1	57.3
74.2	76.8	70.1

*Weaning piglets (n=6) feeding on isocaloric diet, test protein as sole protein source

Functionalities of canola protein & protein products

Wide functionality range:

- Solubility
- Foaming
- Emulsifying
- Heat induced gelling

Intended Use, FDA approved 2 protein products:

- bakery products -2%
- salad dressings -2%
- dairy products -5%
- fruit and vegetable juices and beverages – 10%
- meal replacements & nutrition bars -50%
- Protein powders – 95%
- meat products -2%
- Egg substitutes -60%

GRAS

Protein composition (CRU, NAP or mixed) of the protein product is a key factor – Different than soy or other seed proteins

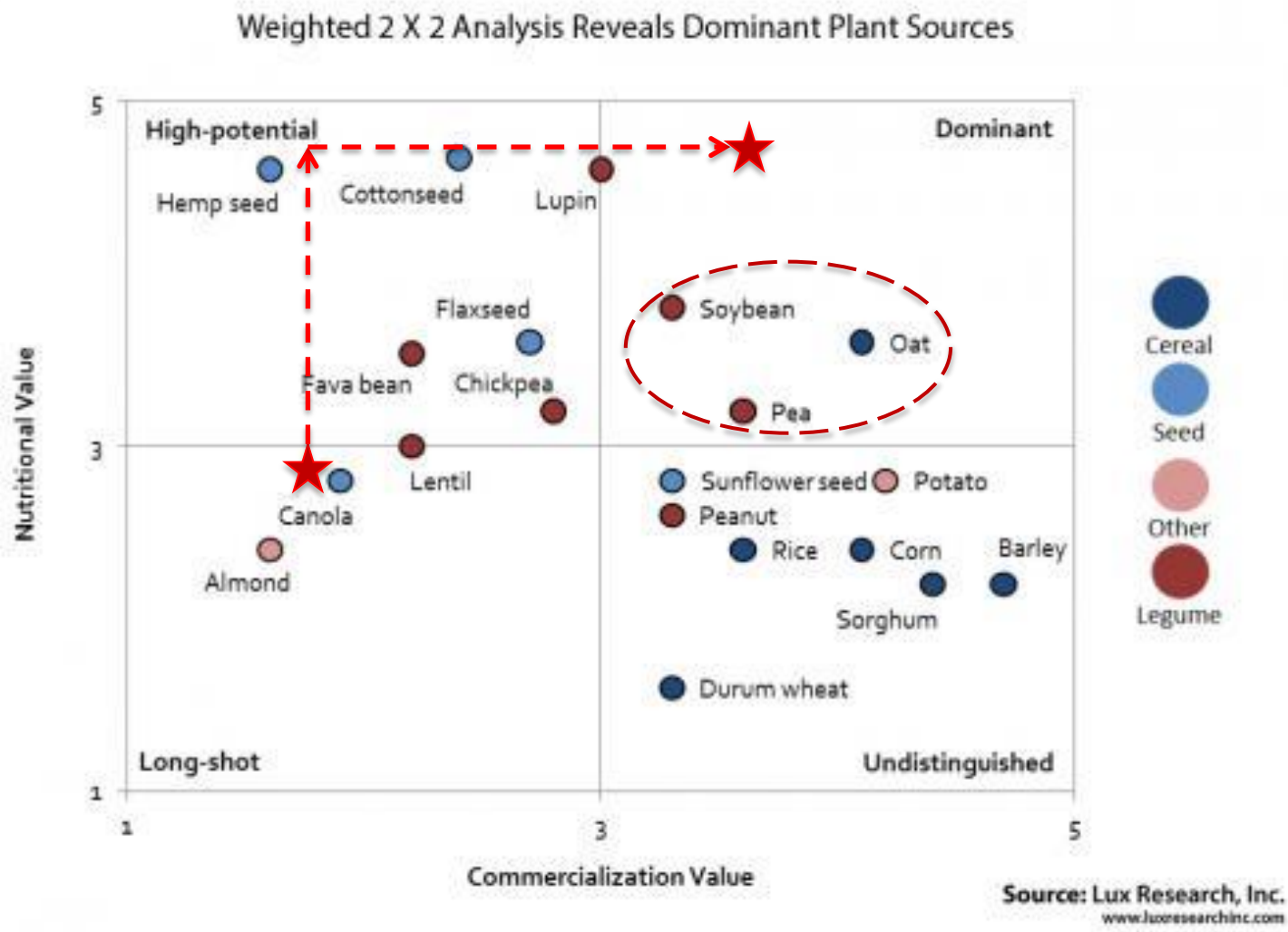
Value beyond amino acid nutrition

Lowering of high blood pressure

↑satiety ↓food intake

	Biological activity and test model	Peptide sequence	Relation to major seed storage protein
2003	• ACEI - *In vitro*, HHL substrate • Antihypertensive activity – SHR model	IY, RIY, VW, VWIS	Cruciferin (IY, VW) Napin (IY, RIY) Ribosomal protein (VWIS)
	• Food intake and gastric emptying using male ddy mice model	RIY	Napin
Pedroche et al., 2004	• ACEI - In vitro, HHL substrate	No peptide sequences reported	Not identified
Wu et al., 2008	• ACEI - In vitro, HHL substrate	VSV, FL	Cruciferin (VSV, FL) Napin (VSV)
Yoshie-Stark et al., 2006	• ACEI - In vitro, HHL substrate • Bile acid binding - In vitro binding of Na cholate • Radical scavenging – In vitro DPPH radical scavenging	No peptide sequences reported	Not identified
Yust et al., 2004	• In vitro pepsin assay and Cell assay with E. coli containing plasmid PT_5 with cDNA coding for HIV-1 proteinase	No peptide sequences reported	Not identified

Predictions for canola protein & protein products



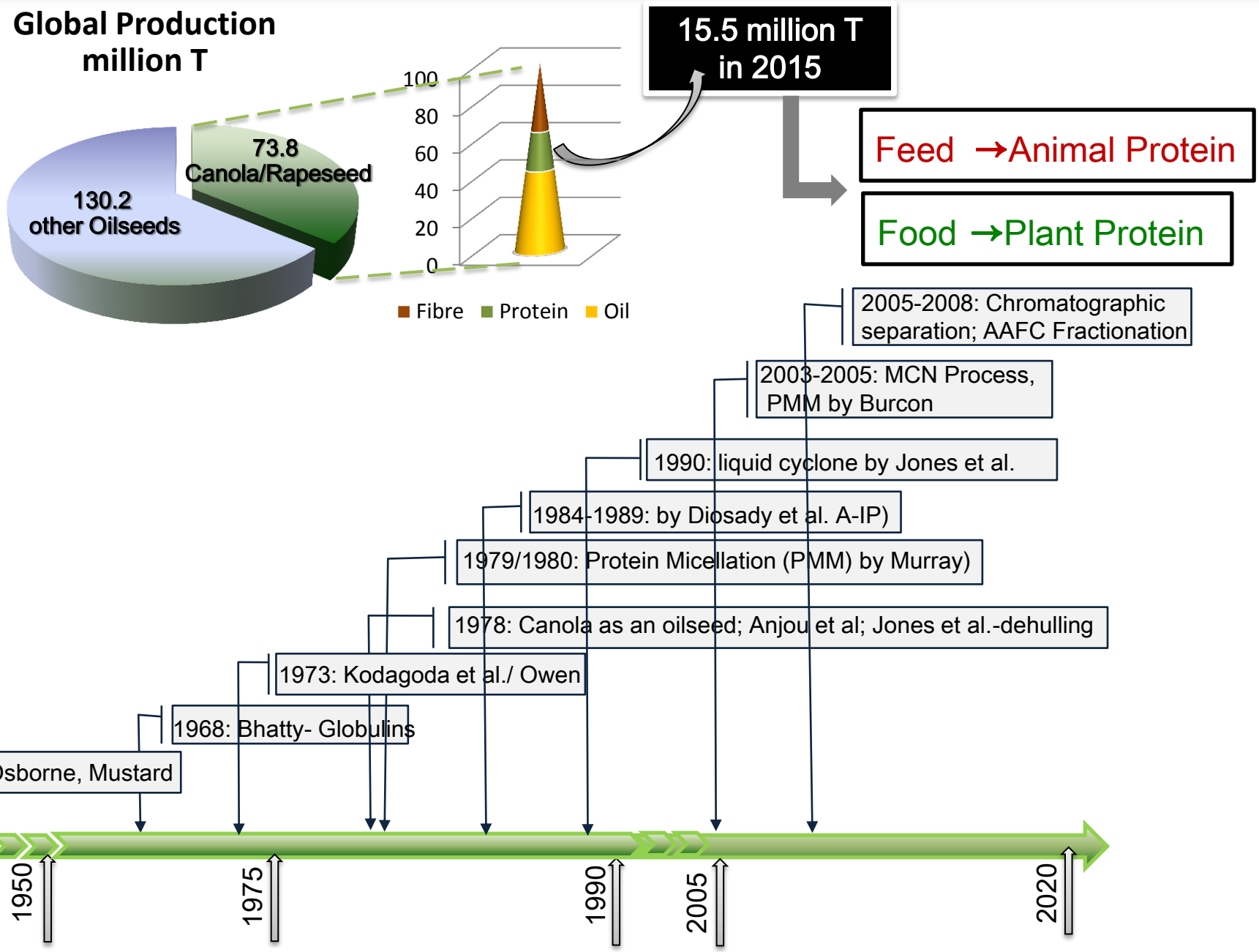
2016

Acknowledgements

Agriculture and Agri-Food Canada
Saskatchewan Canola Development Commission (SaskCanola)
Agriculture Development Fund (ADF) Saskatchewan Ministry of Agriculture

Dr. Dwayne Hegedus and Group, AAFC Saskatoon

Thushan Withana-Gamage, Ph.D.
Pranabendu Mitra, Ph.D.
Suneru Perera, M.Sc.
Sujeema Abeysekara, M.Sc.
Several summer students


Global Production
million T
73.8
Canola/Rapeseed
130.2
other Oilseeds
100
80
60
40
20
0
Fibre
Protein
Oil
15.5 million T
in 2015
Feed →Animal Protein
Food →Plant Protein
2005-2008: Chromatographic separation; AAFC Fractionation
2003-2005: MCN Process, PMM by Burcon
1990: liquid cyclone by Jones et al.
1984-1989: by Diosady et al. A-IP)
1979/1980: Protein Micellation (PMM) by Murray)
1978: Canola as an oilseed; Anjou et al; Jones et al.-dehulling
1973: Kodagoda et al./ Owen
1968: Bhatty- Globulins
Osborne, Mustard
1900
1950
1975
1990
2005
2020

Nutritional studies

In Human:

Protein product: Cruciferin (37%), napin (41%)& LTPI 2.7%)

- True ileal digestibility, 84% (egg 94%, milk 95%) – ^{15}N labelled protein
- Post retention of amino acids is high –relates to high SAA level
- PDCAAS -0.86 – Similar to soy protein isolate - cross-over intervention

Results agree and correlate with piglets and rat studies

13-week rat feeding for toxicity evaluation:

- Napin-rich –12.6 & 14.9 g/kg bw/day male & female
  No Observed adverse effect level (NOAEL) 20%
- Cruciferin rich -11.2 & 14.1 g/kg bw/day, NOAEL 10%

Product Functionalities

Solubility

Protein level %, w/v	Napin isolate			Cruciferin concentrate			Soy protein concentrate			Whey protein isolate		
	p H 4	pH 7	pH 10	pH 4	pH 7	pH 10	pH 4	pH 7	pH 10	pH 4	pH 7	pH 10
0.5	87.6	84.6	90.2	0.0	0.0	8.0	0.0	0.0	0.0	95.1	99.3	96.8
1.0	85.4	81.4	87.1	0.0	4.90	36.5	0.0	0.0	0.0	89.9	98.0	98.1
2.0	84.3	84.4	87.6	9.2	14.0	28.8	0.0	0.0	7.3	91.3	99.4	100

Foaming properties

Protein level %, w/v	Napin isolate			Cruciferin concentrate			Soy protein concentrate			Whey protein isolate		
	p H 4	pH 7	pH 10	pH 4	pH 7	pH 10	pH 4	pH 7	pH 10	pH 4	pH 7	pH 10
Foam capacity, %												
0.5	102.0	204.3	246.0	30.3	37.8	90.7	22.7	22.7	41.6	109.2	109.0	181.6
1.0	204.3	211.2	397.0	28.6	52.5	82.5	45.4	22.6	71.8	196.7	200.5	230.7
2.0	226.9	438.8	423.7	52.9	60.5	67.8	45.4	56.1	71.7	230.7	223.2	313.9
Foam stability, % of original foam												
0.5	69.9	66.6	60.0	0.0	0.0	87.0	0.0	0.0	0.0	96.7	89.7	54.2
1.0	56.6	58.8	72.1	0.0	0.0	95.5	0.0	0.0	57.8	59.6	56.7	55.6
2.0	56.9	78.0	80.3	57.2	62.5	100.0	0.0	53.5	63.3	58.9	57.5	61.1

Amino acid digestion and availability of canola protein products

FOOD	PDCAAS* & LAA	REFERENCE
Buckwheat	80, Leu	Eggum et al., 1999
Quinoa (dried, milled)	109, Lys	FAO/WHO/UNU 1985
* Potato	88, Leu	Jørgensen et al., 2008
* Pea (soaked, dried)	48, Met + Cys	FAO/WHO 1973
* Pea (cooked)	91, Met + Cys	FAO/WHO 1973
*Pea protein (Nutralys®)	82, Met + Cys	Roquette, 2008
Canola protein (napin-r)	92, Phe + Tyr	FDA GRAS Notice 327
*Canola protein (cruciferin-r)	77, Phe + Tyr	FDA GRAS Notice 327
* Whole hempseed	49-53, Lys	House et al., 2010
Hempseed meal	46-51, Lys	House et al., 2010
Hempseed (dehulled)	63-66, Lys	House et al., 2010

Others: Almond - ~23 – 73%; Peanut - ~ 34-70%

*Calculated using reference pattern for 3-10 yr child; LAA – Limiting amino acid

Food and Drug Administration (FDA) 2010

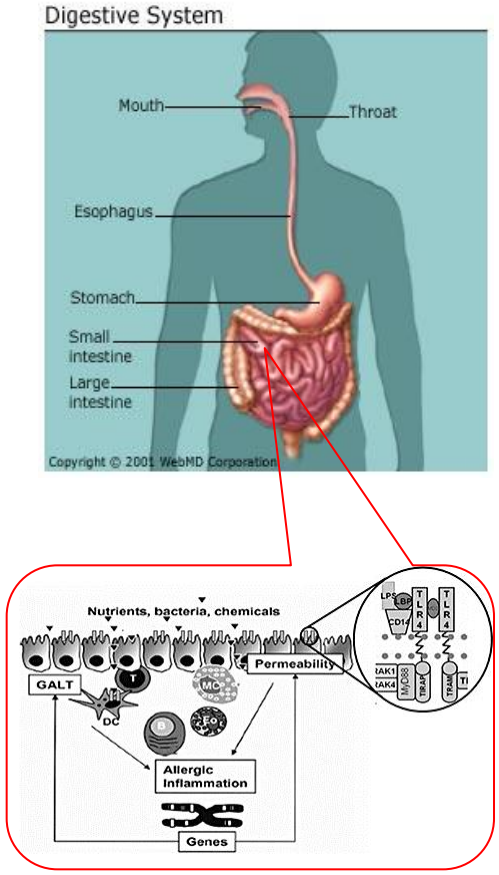
- >3yrs of age

European Food Safety Authority (EFSA) 2013

- "Heavy" adult consumer – 2.2g/kg/bw/d
- 4-6 yr old – 3-4.73 kg/bw/d
- Allergenicity of relative *spp* cannot be excluded
- Similar PDCASS as soy bean protein products
- Sub-chronic toxicological evln of similar rapeseed protein composition

Emulsifying properties

Protein level %, w/v	Napin isolate		Cruciferin concentrate		Soy protein concentrate		Whey protein isolate	
	EAI	ES%	EAI	ES%	EAI	ES%	EAI	ES%
0.5	32.5	5.5	39.5	87.0	60.2	95.5	301.0	109.5
1.0	33.4	2.0	14.4	110.0	ND	ND	ND	ND
2.0	17.3	2.0	20.5	106.3	14.1	107	72.8	113.6



$$AAS\% = \frac{\text{mg of EAA in 1 g test protein}}{\text{mg of same EAA in 1 g of reference protein}} \times 100$$

Lowest EAA ratio is the AAS, 0-1 or 1-100

$$PDCAAS\% = AAS \times \text{true digestibility}$$

PDCAAS: Protein quality based on EAA requirement of human and their ability digest it (fecal)

True protein digestibility, Rat balance method, (1989 Joint FAO/WHO)

DIASS: Protein quality based on EAA requirement of human and their ability digest it (ileal)

Ileal digestibility of each AA, Rat balance method, (2011 Joint FAO/WHO)

Calculated for each EAA and the lowest value is considered 1-100, >100 is truncated