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Potential of Mustard as a Protein Crop

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MUSTARD

- Includes several species of Brassicaceae family:
  Brassica juncea - Oriental & Brown mustard
  Brassica nigra – Black mustard
  Brassica carinata – Ethiopean/Abyssinian mustard
  Sinapis alba – Yellow/white mustard

- Spread around the world
- Canada is one of the largest producers
- Canadian production – 1.6*Yellow*:1*Brown*:1*Oriental*
- ~75% of Canadian production is exported

How do we consume mustard?

A condiment and a Spice – Whole or cracked seed, dry mixes, ground, in wide range of products

As a Paste – Ground seeds, water, vinegar, additives
pungency, consistency

An Oil source – Vegetable oil, very low in SFA, essentially by pressing,
limited to certain cuisines
Essential oil, distillation of volatile oil
Medicinal oil

Ground flour: Heat treated (de-heated) mustard flour, DHMF
- For protein and polysaccharides
- Myrosinase inactivated by heat (110-180°C); no "bite"

A Binder, Emulsifier, Oil & Water absorber, Flavour enhancer, Thickener, Antioxidant & Antimicrobial agent; Mostly *S. alba* is used

Uses of DHMF…

- Processed Meat products
  - Emulsifies fat
  - Binds water and fat - reduce cooking loss (4%)
  - Increases peelability of sausages
  - Reduces lipid oxidation of products –preserves flavour
  - Replaces, milk protein ingredients
  - Reduces production cost

- Sauces and Mayonnaise
  - Emulsifies fat
  - A thickener – replaces polysaccharide gums
  - Replaces egg yolk powder
  - Increases smoothness and texture due to high liquid absorption capacity
  - A natural replacer for tomato solids

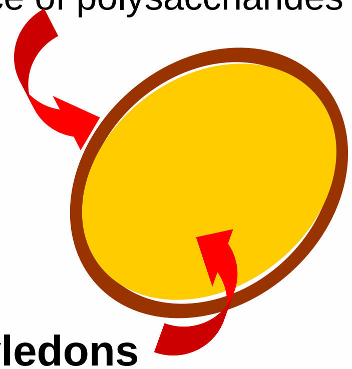
Uses of DHMF…

- Bakery products
  - Improves shelf life
  - YM flour can be used similar to soy flour
  - Improves colour and flavour (gives a hint of mustard?)
  - Can replace egg yolk (1:1) in formulations
  - Provides antioxidant effect

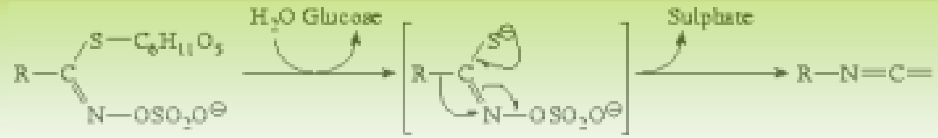
- Processed cheese products
  - Stabilizes consistency
  - Improves sliceability (2-3% inclusion)
  - Reduces stickiness
  - Improves heat stability

Seed composition

Seed coat
Source of polysaccharides - yellow mustard

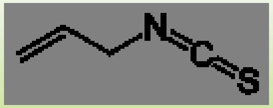


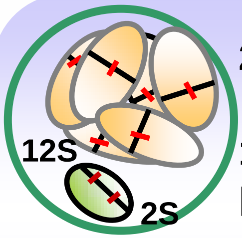
Cotyledons
Source of protein, oil, glucosinolates



S. alba: 4-OH benzylglucosinolate
4-OH benzoic isothiocyanate – non volatile
Gives heat feeling, sweetness in the mouth

***B. juncea/nigra*:** 2-propenyl (allyl) gluc
Allyl isothiocyanate –volatile oil
Strong olfactory, pungent, lachrymatory

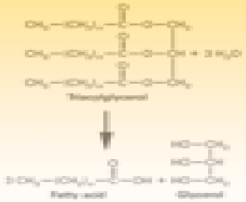


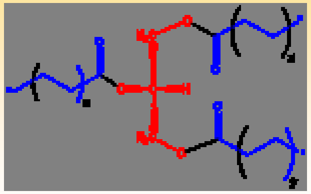


2 Major storage proteins

12 S Cruciferins – High MWT
Major protein

2S napins – Low MWT
Lesser amount
TIA, Allergenic, High thermal stability





FA composition:
Low in SFA, high in MUFA & PUFA
High in Erucic acid

Rich in Vit E
YM - rich in Sitosterol esters
(twice as in *B. napus or juncea)*

Fatty acid composition

Fatty acid	Oriental	Brown	Yellow	Canola
16:0 Palmitic	2.9-3.1	2.9-3.1	2.6-2.7	3.5
18:0 Stearic	1.5-1.6	1.4-1.5	1.0-1.1	1.5
20:0 Arachidic	0.8-0.9	0.9	0.6	0.6
22:0 Behenic	0.4-0.5	0.2-0.3	0.5	0.3
24:0 Lignoceric	0.3	0.4-0.5	0.3	-
Total SFA	*5.9-6.4*	*5.8-5.9*	*5.0-5.2*	5.9
16:1 Palmitoleic	0.2	0.2	0.2	0.2
18:1 Oleic	21.4-25.2	21.5-24.0	23.2-25.6	60.1
20:1 Gadoleic	12.3-13.4	12.2-13.3	11.0-11.4	1.4
22:1 Erucic	18.4-22.8	21.0-22.8	33.4-38.1	0.2
24:1 Nervonic	1.2-1.4	1.1-1.2	2.1-2.3	-
Total MUFA	*53.5-62.8*	*56.0-61.5*	*69.9-77.6*	61.9
18:2 Linoleic	21.7-23.6	20.8-21.3	9.0-9.8	20.1
18:3 Linolenic	11.1-11.8	12.5-12.7	10.0-10.9	9.6
20:2 Eicosadienoic	1.0-1.1	0.9-1.0	0.3	-
22:2 Docosadienoic	0.4-0.5	0.3-0.5	0.3-0.4	-
Total PUFA	*34.2-37.0*	*34.5-35.5*	*19.6-21.3*	29.7

Source: Canadian Grain Commission & Canola Council Canada

Seed Type		Protein content, % (dwt)	Oil content, % (dwt)
Brassica juncea			
AC Vulcan	Whole seed	28.6	45.2
	Cotyledons	29.7	51.2
	Hulls	19.2	17.3
Duchess	Whole seed	28.6	46.2
	Cotyledons	31.7	47.2
	Hulls	16.5	10.3
Sinapis alba			
AC Pennant	Whole seed	34.5	35.9
	Cotyledons	35.6	31.8
	Hulls	18.9	10.5
Andante	Whole seed	39.5	32.7
	Cotyledons	41.5	33.9
	Hulls	19.6	8.3

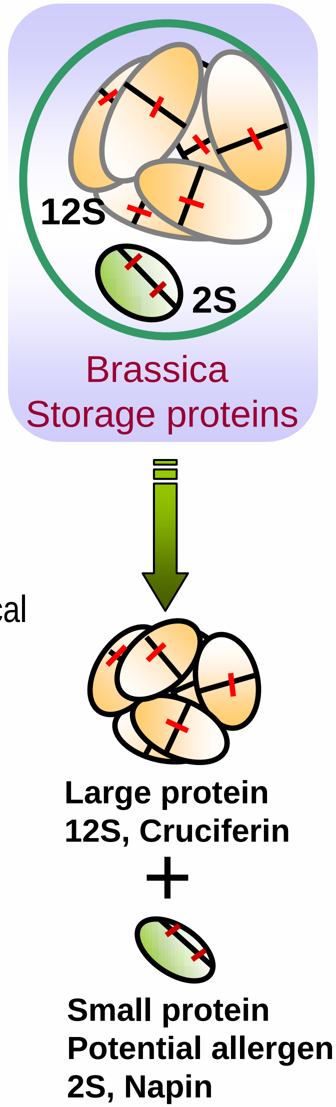
B. napus	whole seed	25.2	41.0
Soy bean	whole seed	42.6	22.0

Seed storage proteins of mustard

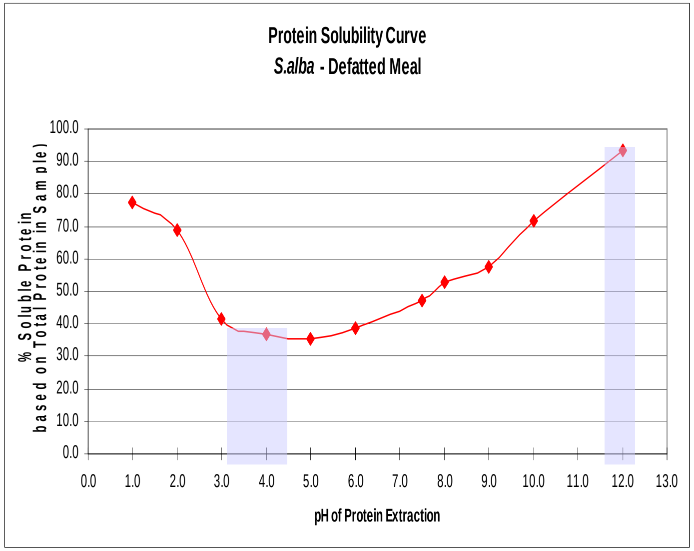
- Two major storage proteins – 11S cruciferins & 2S napins
  - Different in molecular assemblies, molecular masses, biochemical properties, biological activities
  - Has similarities with other crucifer proteins such as canola

- Current understanding on proteins
  - No estimate on the potential of germplasm to provide 11S and 2S
  - No clear understanding on the value of 11S and 2S proteins of crucifers
  - Role of other seed components on proteins e.g., fibre; nutritional & technological
  - Both *B. juncea* & *S. alba* contains allergenic proteins in the 2S fraction

- Nutritional quality
  - Fairly balanced amino acid composition
  - High levels of sulfur-containing amino acids & lysine than legumes or cereals
  - High protein biological value in humans as high as milk proteins (*B. napus* protein isolate 11S+2S, Bos *et al.* 2007)

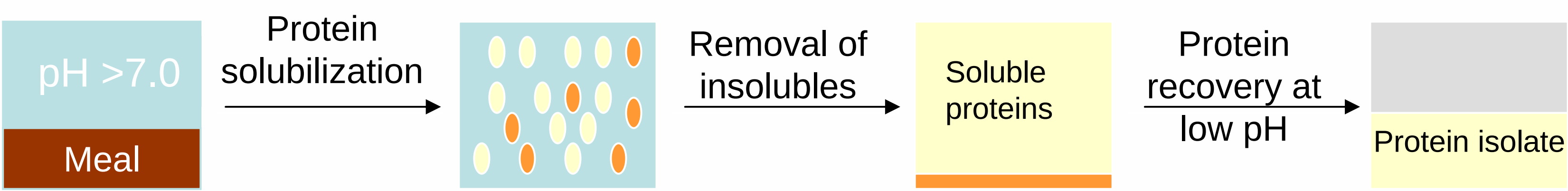


What technologies are available for protein recovery?



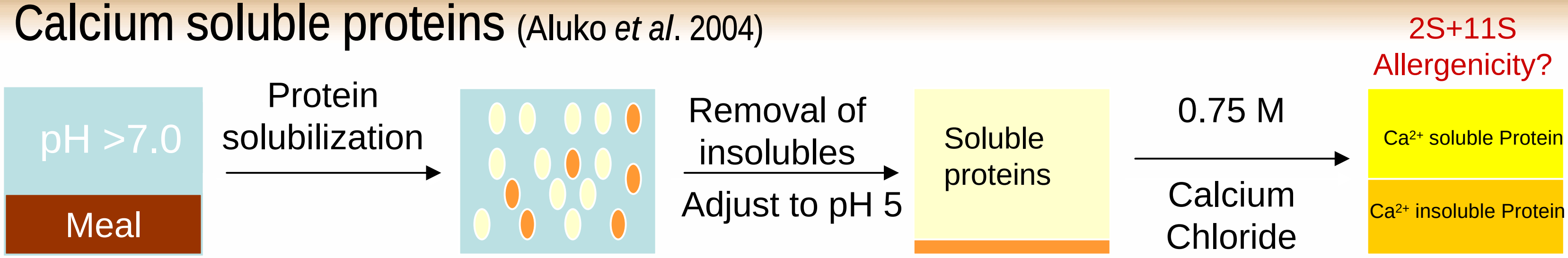
- Need to use oil removed meal to recover proteins.
- A fraction of Crucifer seed proteins are soluble at low pHs such as 3 and 4.
- Types of protein soluble at these pHs are different

Alkali solubilization-Isoelectric precipitation (Diosady *et al*. 2003, 2006)

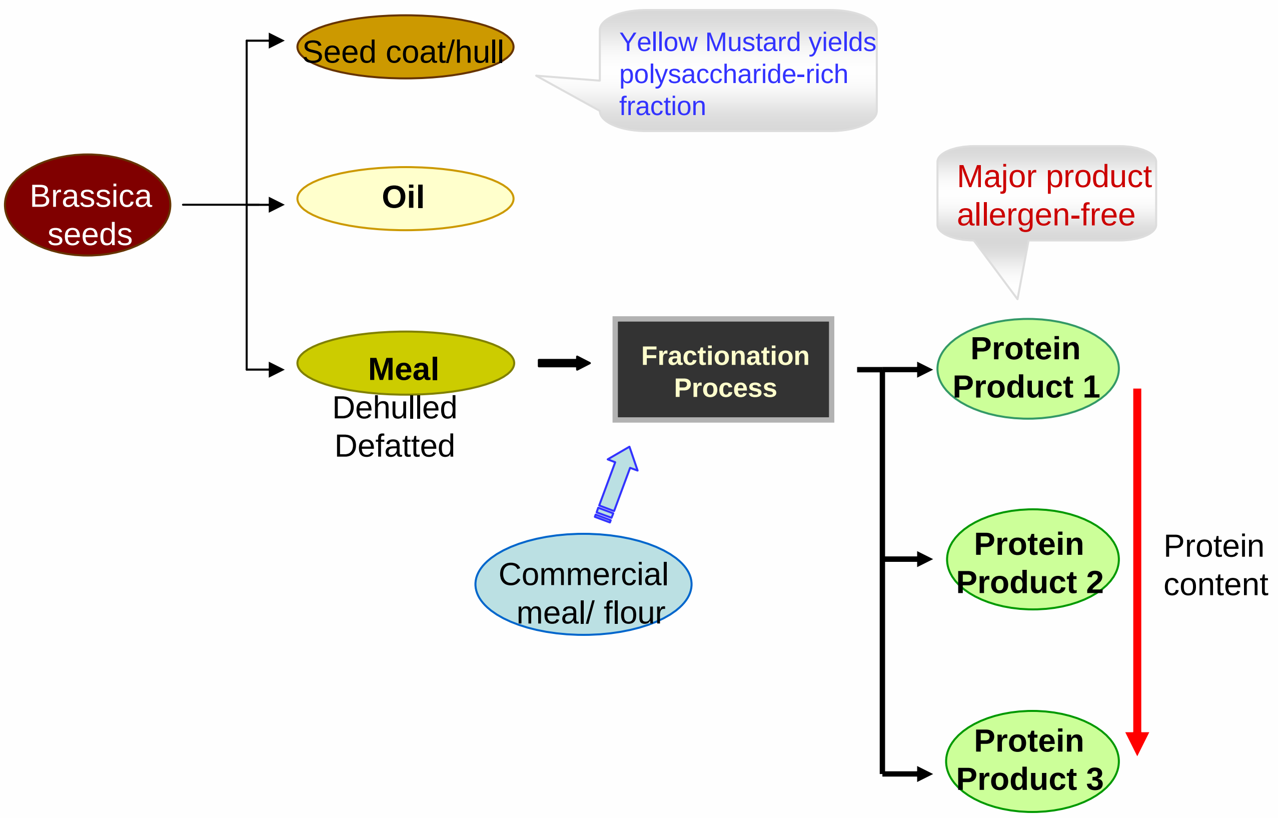


2S+11S
Allergenicity?

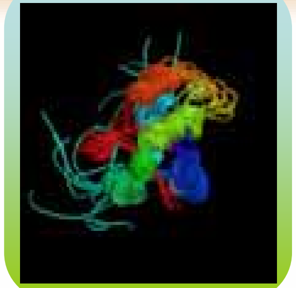
Calcium soluble proteins (Aluko *et al*. 2004)



AAFC Fractionation Model for Brassica Oilseed Proteins (Wanasundara, unpublished)



Allergenicity of mustard proteins



- Most of the research is from Europe – young children (ranked 4th after egg, peanut & cow milk)
  S. alba – *Sin a* 1
  B. juncea - *Bra j* 1E - Both are essentially napins
- Proteins of 14 to16 kDa, heat stable, resistant to trypsin digestion
- EU Directive 2003/89/EC- a priority allergen, must be declared in foods

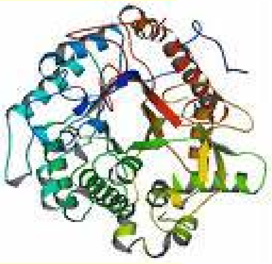
Allergenic protein *Sin a* 1 content of Yellow Mustard as determined by sandwich-ELISA (Shim & Wanasundara, 2007)

YM	Total protein (mg/g)[c]	*Sin a* 1 (mg/g)[c]	*Sin a* 1 content as % of total protein[d]
AC Base	244.3±11.8b	1.80±0.29c	0.737
AC Pennant	324.5±9.2a	2.32±0.28b	0.715
Andante	388.7±19.7a	2.94±0.50a	0.756
HS3	176.4±11.2d	0.82±0.09d	0.465
HS4	181.8±13.5d	0.82±0.08d	0.451
HS5	209.6±13.8c	1.08±0.12d	0.515
Range (mg/g)	176.4–388.7	0.82–2.94	0.451-0.756

[a] Sandwich-ELISA was performed
[b] The defatted ground YM powders were slurried in PBS and used for the detection of *Sin a* 1.
[C] All values are means of triplicate determinations. Values followed by the same letters are not significantly different at $p < 0.005$.
[d] Mean values of total protein and *Sin a* 1 was used for calculation

Any other useful proteins?

Myrosinase



- Enzyme that catalyses the hydrolysis of Glucosinoates to Isothiocyanates.
- To generate allyl isothiocyanates from *B. juncea* myrosinase has to be active.
- Needed to generate the “bite” as a spice and also for the pesticide applications
- YM myrosinase is more efficient and active than *B. juncea*

Mustard trypsin inhibitors (MTI-1, MTI-2)

- Similar to rapeseed trypsin inhibitor, high affinity towards trypsin than chymotrypsin
- Cystein-rich 2S proteins (8 Cys residues /molecule)
- MTI-1; 18 kDa heat labile (80°C), MTI-2; ~7kDa, heat stable

Short Peptides conserved within large proteins

- Peptides with bioactivities

  e.g. antioxidative, antihypertensive, Ca-absorbing, etc.

Opportunity for mustard…

As a crop

- Not a new crop
- SK produces >80% Canadian mustard, 50% of the world production

Mainly yellow mustard is processed in Canada

- YM seed is a rich source of three valuable macro components in food processing; polysaccharide gums, oil and proteins.
- Provides variety of technologically important functions
- Can they compete with proteins of Soy, Egg or Milk?

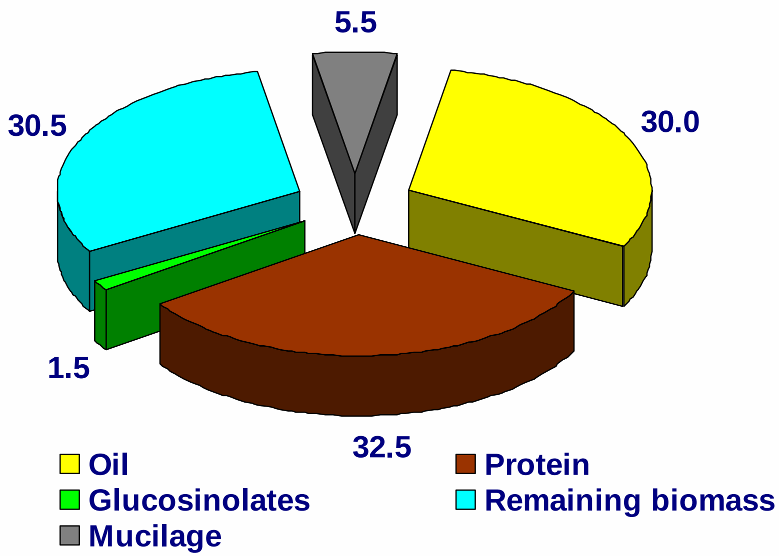
Already have uses in human food chain: No GMO issues

Diverse products may be the key to increase production

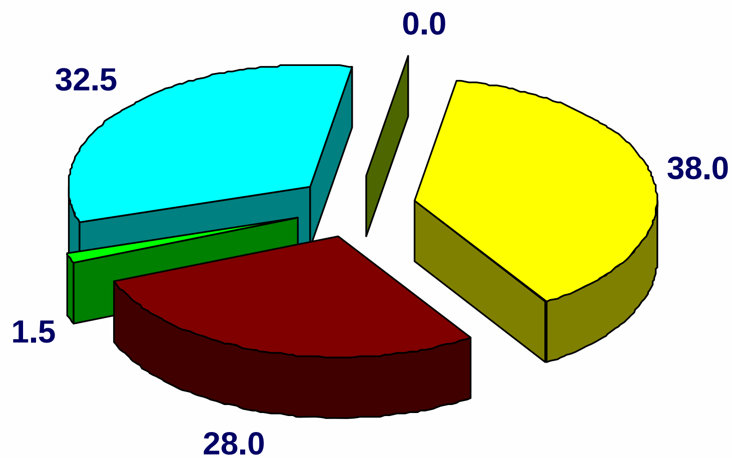
- New products/uses beyond whole seed applications
- Need separate approaches for YM and O/B mustard
  – different in composition thus the potential

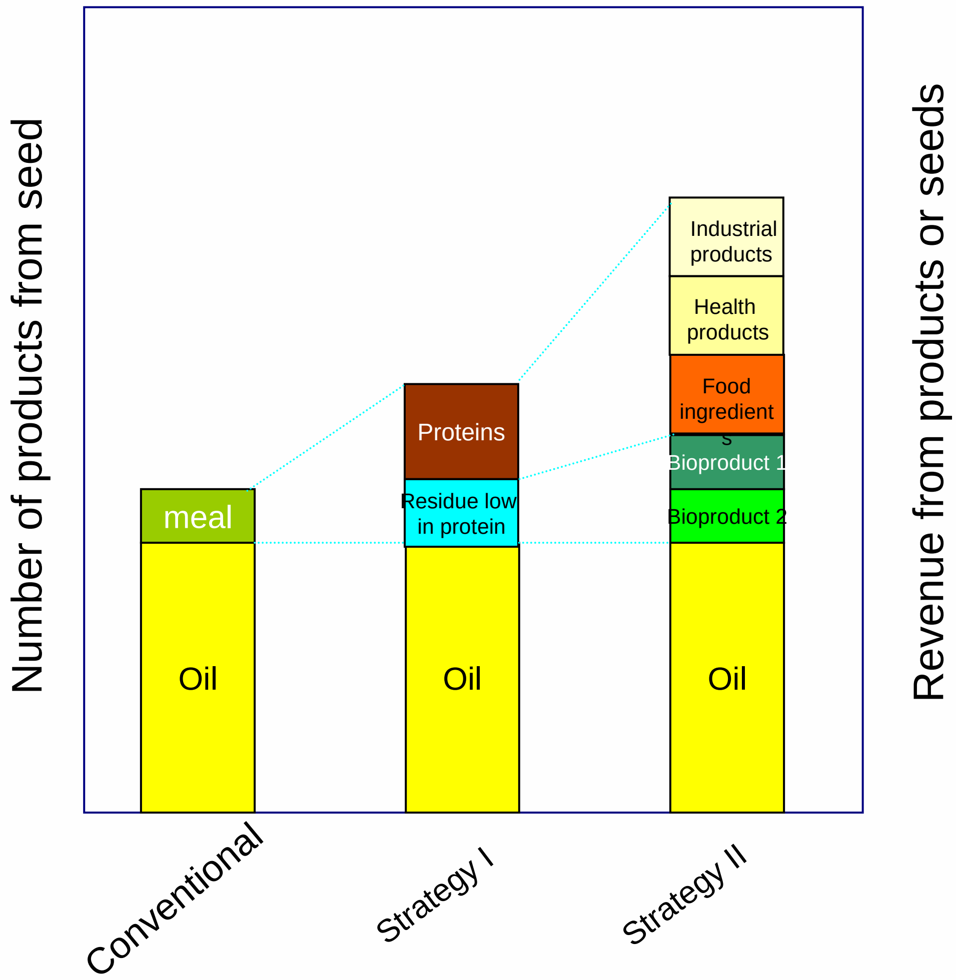
Opportunity for mustard...

Yellow mustard



Oriental mustard





Mustard Proteins - Scientific & Technical Know-how

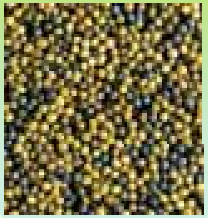
Scientific Knowledge

Seed chemistry

Protein Biochemistry
- Storage proteins
- Minor proteins

Health & nutrition related properties

Technologically important properties



Ingredient Development

Protein recovery methods
- Quality & Quantity

Improving desirable properties
- Technological/functional
- Nutritional

Ingredients with diverse functions
- Bulk protein -unmodified
- Hydrolyzed protein
- Acylated proteins
- Phosphorylated proteins
- Glycosylated proteins

Consumer products

Selection of suitable product platform

Food **Regular & Functional**
- – Beverage
- Dessert-type
- Energy bars
- Flavour enhancers
- Extruded products

Feed – Aqua feed

Non food, non feed –
- Industrial adhesives/glues
- Foams

Thank You

Canada

Agriculture and Agri-Food Canada
Agriculture Development Fund (SK)
Saskatchewan Mustard Development Commission