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Potential of Mustard as a Protein Crop

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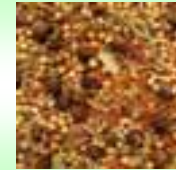
MUSTARD

- Includes several species of Brassicaceae family:
 - Brassica juncea* - Oriental & Brown mustard
 - Brassica nigra* – Black mustard
 - Brassica carinata* – Ethiopian/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

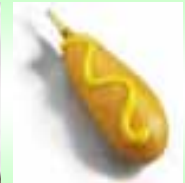


How do we consume mustard?

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

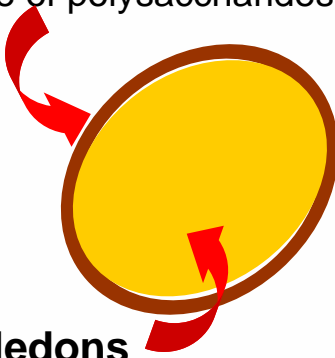
- Improves heat stability



Seed composition

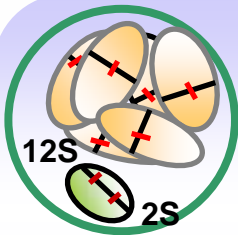
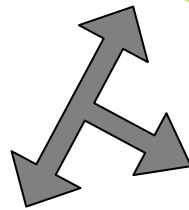
Seed coat

Source of polysaccharides - yellow mustard



Cotyledons

Source of protein, oil, glucosinolates

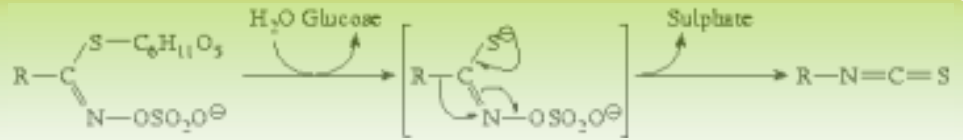


2 Major storage proteins

12S Cruciferins – High MWT
Major protein

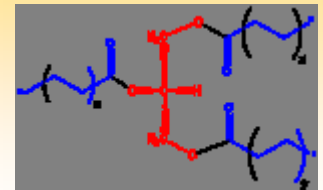
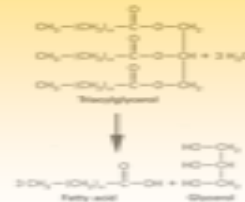
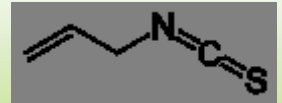
2S napins

– Low MWT
Lesser amount
TIA, Allergenic, High thermal stability



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



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)
<i>Brassica juncea</i>			
<u>AC Vulcan</u>	Whole seed	28.6	45.2
	Cotyledons	29.7	51.2
	Hulls	19.2	17.3

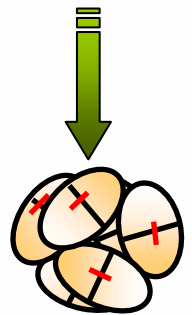
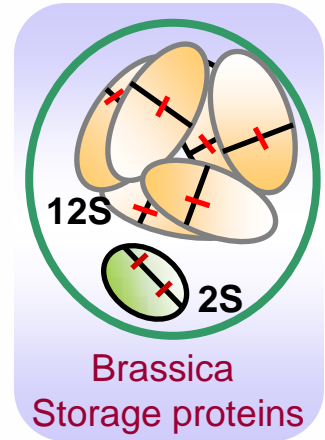
<u>Duchess</u>	Whole seed	28.6	46.2
	Cotyledons	31.7	47.2
	Hulls	16.5	10.3
<i>Sinapis alba</i>			
<u>AC Pennant</u>	Whole seed	34.5	35.9
	Cotyledons	35.6	31.8
	Hulls	18.9	10.5

<u>Andante</u>	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)



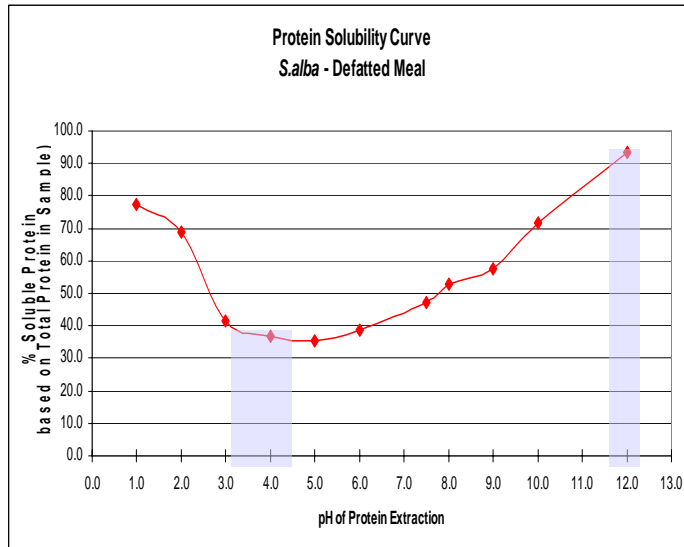
Large protein
12S, Cruciferin

+



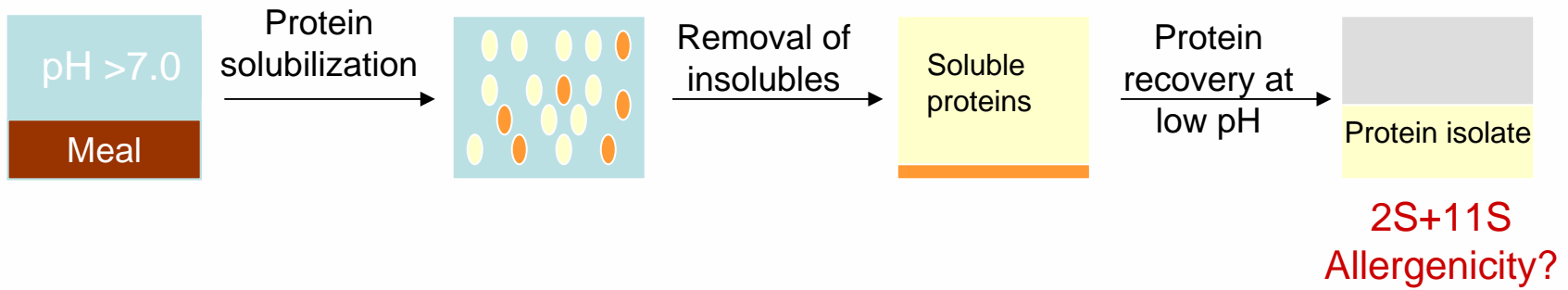
Small protein
Potential allergen
2S, Napin

What technologies are available for protein recovery?

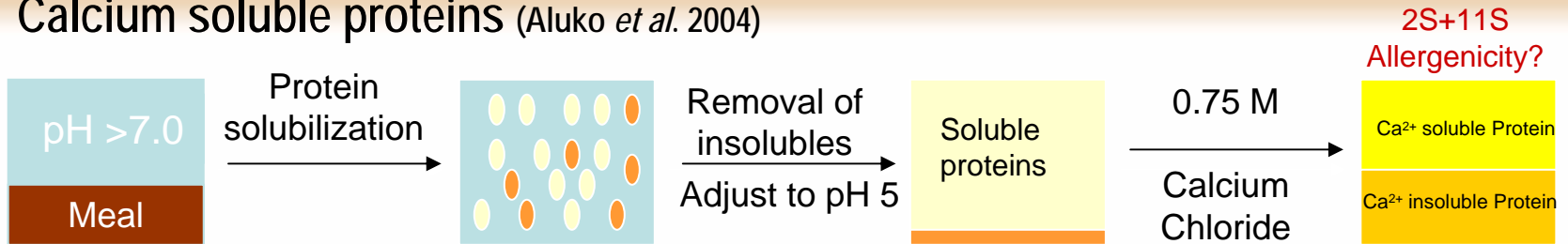


- Need to use oil removed meal to recover proteins.
- A fraction of Crucifer seed proteins are soluble at low pHs such as 3 and 4.
- Types of protein soluble at these pHs are different

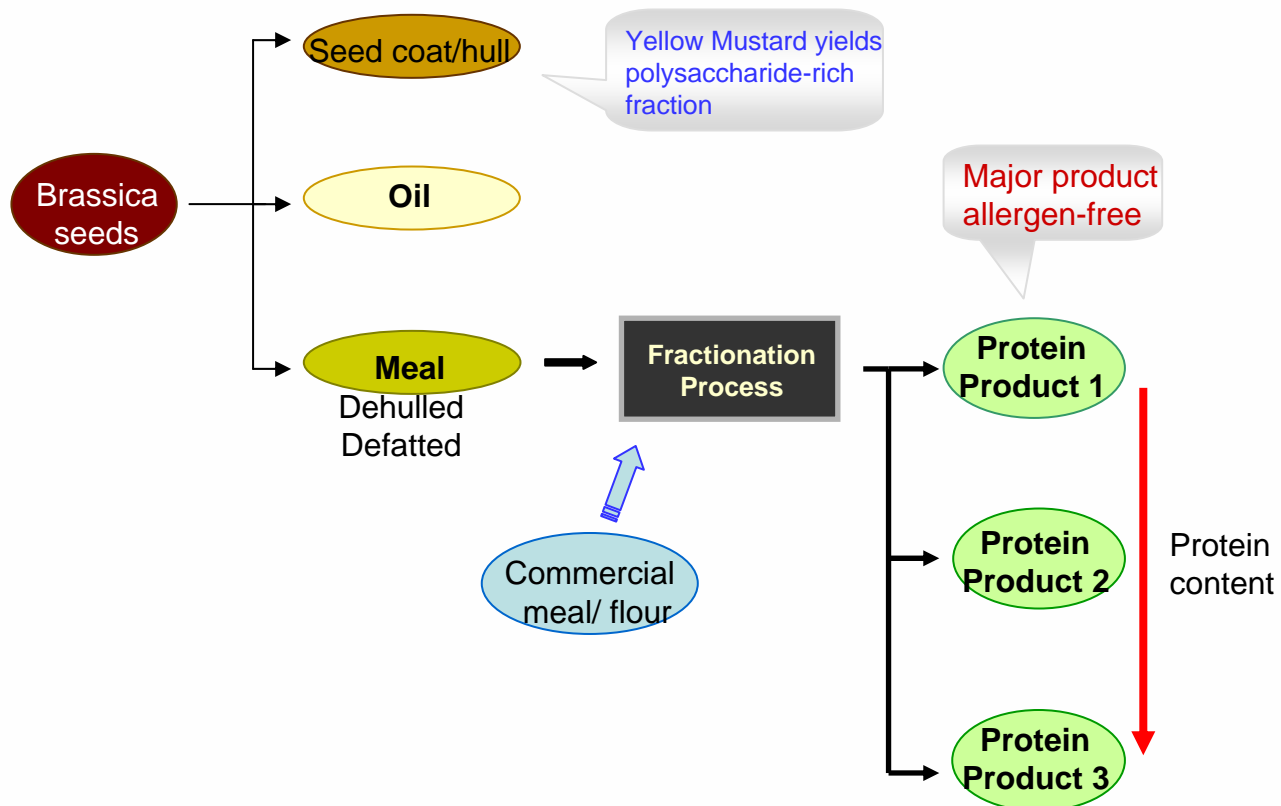
Alkali solubilization-Isoelectric precipitation (Diosady *et al.* 2003, 2006)



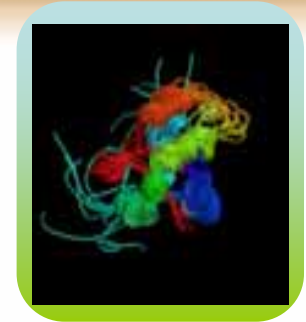
Calcium soluble proteins (Aluko *et al.* 2004)



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 to 16 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	<i>Sin a 1</i> (mg/g) ^c	<i>Sin a 1</i> 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 Glucosinates 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?



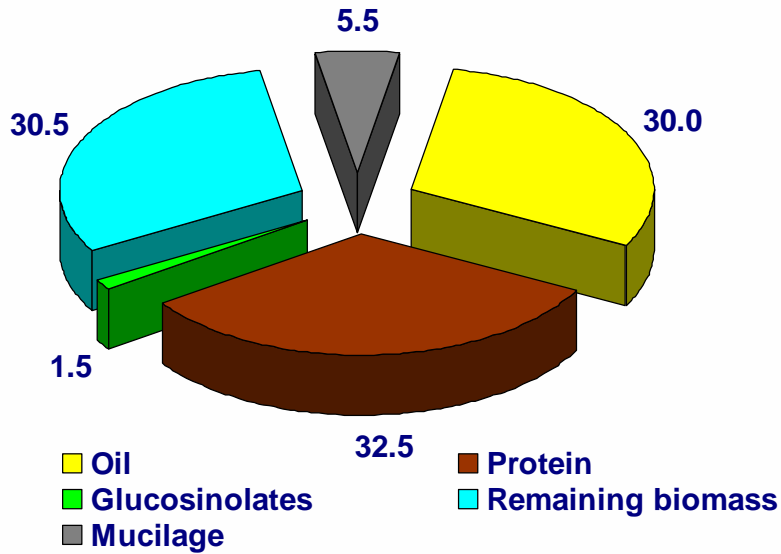
Already have uses in human food chain: No GMO issues

Diverse products may be the key to increase production

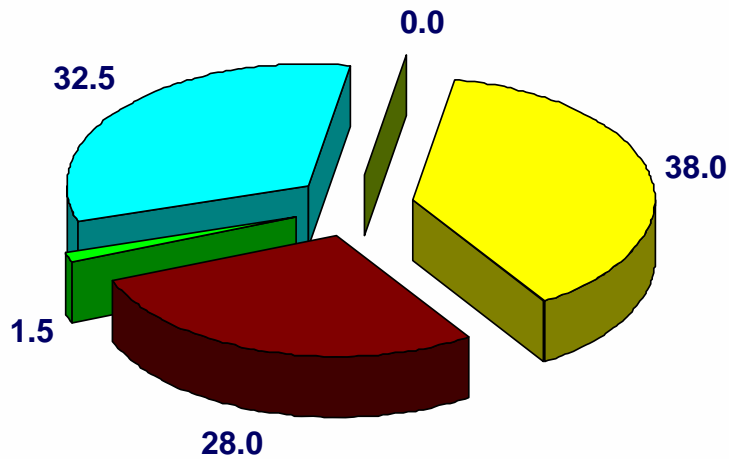
- New products/uses beyond whole seed applications
- Need separate approaches for YM and O/B mustard
 - different in composition thus the potential

Opportunity for mustard...

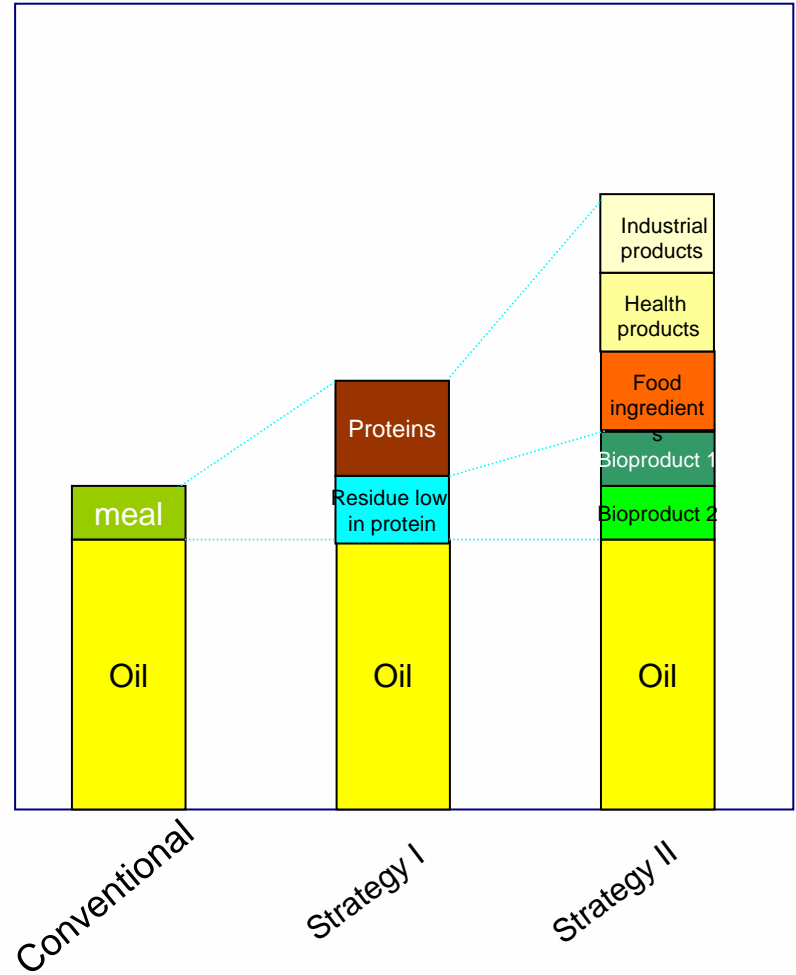
Yellow mustard



Oriental mustard



Number of products from seed



Revenue from products or seeds

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