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 – 1.6:1 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

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

Source: Canadian Grain Commission & Canola Council Canada
<table>
<thead>
<tr>
<th>Seed Type</th>
<th>Protein content, % (dwt)</th>
<th>Oil content, % (dwt)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brassica juncea</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC Vulcan</td>
<td>Whole seed</td>
<td>28.6</td>
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<tr>
<td></td>
<td>Cotyledons</td>
<td>29.7</td>
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<tr>
<td></td>
<td>Hulls</td>
<td>19.2</td>
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<tr>
<td>Duchess</td>
<td>Whole seed</td>
<td>28.6</td>
</tr>
<tr>
<td></td>
<td>Cotyledons</td>
<td>31.7</td>
</tr>
<tr>
<td></td>
<td>Hulls</td>
<td>16.5</td>
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<tr>
<td><strong>Sinapis alba</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC Pennant</td>
<td>Whole seed</td>
<td>34.5</td>
</tr>
<tr>
<td></td>
<td>Cotyledons</td>
<td>35.6</td>
</tr>
<tr>
<td></td>
<td>Hulls</td>
<td>18.9</td>
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<tr>
<td>Andante</td>
<td>Whole seed</td>
<td>39.5</td>
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<tr>
<td></td>
<td>Cotyledons</td>
<td>41.5</td>
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<tr>
<td></td>
<td>Hulls</td>
<td>19.6</td>
</tr>
<tr>
<td><strong>B. napus</strong></td>
<td>whole seed</td>
<td>25.2</td>
</tr>
<tr>
<td><strong>Soy bean</strong></td>
<td>whole seed</td>
<td>42.6</td>
</tr>
</tbody>
</table>
**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?

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

- 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.

**Protein Solubility Curve**

<table>
<thead>
<tr>
<th>pH of Protein Extraction</th>
<th>% Soluble Protein based on Total Protein in Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1.0</td>
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<tr>
<td>2.0</td>
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<td>12.0</td>
<td>12.0</td>
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<tr>
<td>13.0</td>
<td>13.0</td>
</tr>
</tbody>
</table>

**Meal**

- pH >7.0
- Protein solubilization
- Removal of insolubles
- Soluble proteins recovery at low pH
- Protein isolate

2S+11S
Allergenicity?
**Calcium soluble proteins** (Aluko et al. 2004)

- pH >7.0
- Protein solubilization
- Meal

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

- Brassica seeds
- Oil
- Meal (Dehulled Defatted)

**Fractionation Process**

- Commercial meal/ flour
- Protein Content

**Protein Products**

1. Protein Product 1
2. Protein Product 2
3. Protein Product 3

Yellow Mustard yields polysaccharide-rich fraction

Major product allergen-free

2S+11S Allergenicity?
Allergenic protein \(\text{Sin a 1}\) content of Yellow Mustard as determined by sandwich-ELISA

(Shim & Wanasundara, 2007)

<table>
<thead>
<tr>
<th>YM</th>
<th>Total protein (mg/g)(^c)</th>
<th>(\text{Sin a 1}) (mg/g)(^c)</th>
<th>(\text{Sin a 1}) content as % of total protein(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC Base</td>
<td>244.3±11.8b</td>
<td>1.80±0.29c</td>
<td>0.737</td>
</tr>
<tr>
<td>AC Pennant</td>
<td>324.5±9.2a</td>
<td>2.32±0.28b</td>
<td>0.715</td>
</tr>
<tr>
<td>Andante</td>
<td>388.7±19.7a</td>
<td>2.94±0.50a</td>
<td>0.756</td>
</tr>
<tr>
<td>HS3</td>
<td>176.4±11.2d</td>
<td>0.82±0.09d</td>
<td>0.465</td>
</tr>
<tr>
<td>HS4</td>
<td>181.8±13.5d</td>
<td>0.82±0.08d</td>
<td>0.451</td>
</tr>
<tr>
<td>HS5</td>
<td>209.6±13.8c</td>
<td>1.08±0.12d</td>
<td>0.515</td>
</tr>
</tbody>
</table>

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 \(\text{Sin a 1}\).

\(^c\) 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 \(\text{Sin a 1}\) 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
Oriental mustard

Yellow mustard

Opportunity for mustard...

Number of products from seed

Revenue from products or seeds

Conventional

Strategy I

Strategy II

Bioproduct 1

Bioproduct 2

Industrial products

Health products

Food ingredients

Oil

Oil

Oil

Meal

Residue low in protein

Proteins
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