Effects of Oil Extraction on Functional Properties of Protein in Pennycress (*Thlaspi arvense* L.) Seed and Press Cake

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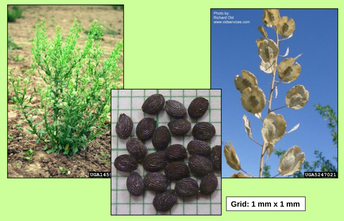
INTRODUCTION

- Pennycress (*Thlaspi arvense* L.) is a common agricultural weed in temperate North America; also known as fanweed, frenchweed, or stinkweed.
- Advantages: high seed yield, high oil content in seeds, suitable for two-crop rotation with soybeans [1].
- Seed contains 36% oil, which has erucic acid as major fatty acid (38%) [1].

Pennycress Plant, Pods, and Seeds



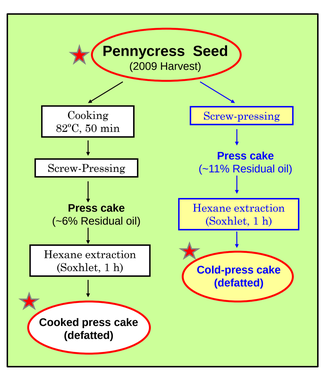
- Pennycress is being developed as an oilseed crop for biodiesel production [2].
- Biodiesel from pennycress oil had high cetane number, excellent low-temperature properties, and met ASTM D6751 specifications [2].
- Defatted seed meal has potential use as biofumigant for horticultural crops [3].
- Defatted seed meal, after fast pyrolysis, produces liquid fuel intermediates suitable for jet fuel formulations [4].
- Currently, there is little information on the properties of pennycress seed protein. These properties should be examined to identify possible value-added uses of the seed protein.

OBJECTIVES

- Determine the effects of oil processing conditions (i.e., cold-pressing or seed cooking) on functional properties of pennycress seed protein
- Identify potential applications of proteins in pennycress seed and press cake

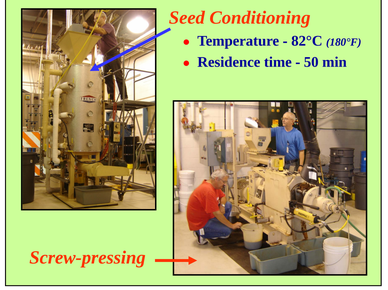
METHODOLOGY

Materials and Oil Processing



METHODOLOGY

Oil Processing of Pennycress



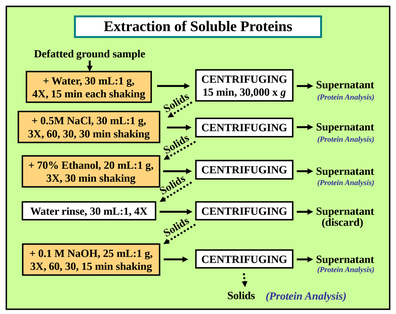
Proximate Analyses

- Moisture - AOCS Ba 2a-38 [5]
- Crude protein (%N x 6.25) - AOCS Ba 4e-93 [5]
- Crude oil - AOCS Am 5-04 [5]

Determination of Soluble Classes

- Method: Hu and Esen [6], modified
- Determined the water-, saline-, ethanol-, and alkali-soluble protein classes in pennycress seed meal and press cakes

Extraction of Soluble Proteins



SDS-PAGE

- Method: Wu and Hojilla-Evangelista [7]
- Sample amount - 4 mg protein/mL in 500 µL of sample buffer *[42 nM Tris-HCl (pH 6.8), 2% SDS, 7% glycerol, 4.4% β-mercaptoethanol, 5M urea].*
- Sample load volume - 15 µL
- Gel - Bis-Tris NuPAGE pre-cast 4-12% gradient gel (Invitrogen Corp., Carlsbad, CA).
- MW standards - Pre-stained broad range SDS-PAGE protein standards *(6.5 -196 kDa)*

Protein Functionality Tests

Ground pennycress seeds were first defatted by 5-6 cycles of hexane extraction at 25°C until residual oil content was < 0.5% (db). Ground press cakes were already at target oil content.

Solubility

- Method: Balmaceda *et al.* [8]
- Sample concentration - 10 mg protein/mL
- pH levels - 2.0, 4.0, 5.5, 7.0, 8.5, and 10.0
- Protein analysis - combustion method

Protein Functionality Tests (continued)

Foaming properties

- Method: Myers *et al.* [9]
- Sample concentration - 10 mg protein/mL
- Determined at pH 7.0 and pH 10.0
- **Foam capacity** - volume (mL) of foam produced in 1 min; **foam stability** - foam remaining (%) after standing for 15 min.

Emulsifying properties

- Method: Wu *et al.* [10]
- Emulsion preparation - 2 mL corn oil + 6 mL protein solutions (1 mg protein/mL); homogenizing at 20,000 rpm for 1 min.
- Calculated emulsification activity index (EAI, in m^2/g) and emulsion stability index (ESI, in min.)

RESULTS

Moisture, Oil, and Protein Contents. Evangelista [1] reported that field pennycress had 9.5% moisture and 32.9% (db) oil. Our ground pennycress seed had 6.1% moisture, 18.9% (db) crude protein, and 30.7% (db) crude oil, which are closest to values reported for sunflower. After hexane-defatting, oil content of the seed meal decreased notably, as would be expected (Table 1).

Table 1. Moisture, Oil, and Protein Contents of Defatted Ground Pennycress Seeds and Press Cakes (PC)

Sample	Moisture %	Crude Protein % (db)	Crude Oil % (db)
Pennycress seed, ground	9.6	33.4	0.34
Cold-pressed PC	8.9	35.1	0.16
Cooked-pressed PC	6.2	35.1	0.21

Soluble Protein Classes. Untreated, ground pennycress seed and cold-pressed cake had very similar distributions of soluble protein classes, with water- and NaCl-soluble fractions accounting for 42% of total protein (Table 2). In the cake from cooked-pressed seed, these protein fractions were much less, but amounts of NaOH-soluble protein and that left in spent solids were greater than in the seed or cold-pressed cake. No ethanol-soluble proteins were detected in all the samples. Freeze-dried saline-soluble extracts from all samples had the highest protein purities among the soluble fractions (Table 3).

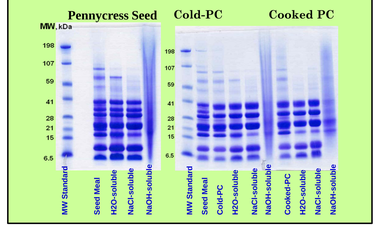
Table 2. Soluble Protein Classes in Pennycress Seed and Press Cakes (PC)

Solvent for Extraction or Sample Fraction	Amount of Protein (% of Total Protein)		
	Pennycress Seed	Cold-PC	Cooked PC
Water	19.4	22.5	13.9
0.5M NaCl	23.0	20.7	17.4
70% Ethanol	0.0	0.0	0.0
0.1M NaOH	4.6	4.7	11.2
Spent Solids	23.2	24.3	33.2
Unaccounted	29.8	27.8	24.3

RESULTS

SDS-PAGE. In reducing gel, pennycress seed protein showed twelve bands, with MW ranging from ca. 100 to < 6.5 kDa, with the darkest bands distributed between 6.5-41 kDa (Fig. 1). These dark bands were also present in the water- and NaCl-soluble fractions. Band patterns for cold-pressed cake and its soluble fractions were similar to those of the untreated seed. In the cake from cooked/pressed seed and its extracts, more higher-MW bands (>59kDa) were detected, while a few others became absent or lighter-colored. Most notable were the distinct bands in the NaOH fraction. These results indicated that heat during the cooking stage had detrimental effects on the pennycress protein.

Fig. 1. SDS-PAGE Band Patterns of Protein in Ground Pennycress Seeds and Press Cakes (PC)



Solubility Profile. Pennycress seed meal protein showed poor solubility at pH 2-10 (Fig. 2), being least soluble at pH 4 and most soluble at pH 10. Protein from both press cakes showed similar solubility behavior, but the cold-press cake protein had slightly higher soluble protein amounts, especially at pH 10.

Fig. 2. Solubility Profiles of Protein in Pennycress Seed Meal and Press Cakes

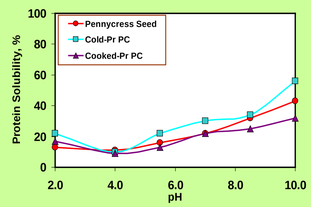


Table 3. Protein Contents of Freeze-dried Soluble Proteins in Pennycress Seed and Press Cakes (PC)

Protein Fraction	Crude Protein Content (%)		
	Pennycress Seed	Cold-PC	Cooked PC
Water-soluble	69.3	81.6	73.1
NaCl-soluble	94.1	88.9	94.1
NaOH-soluble	54.4	58.2	78.3

Acknowledgment

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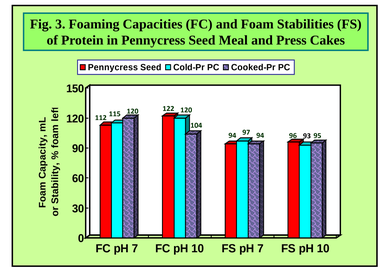
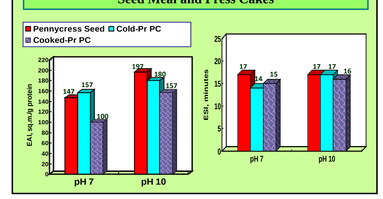
Foaming Properties. Protein in pennycress seed and press cakes produced substantial foam that were also highly stable (Fig. 3). These foaming properties are very comparable to that of soy protein (135 mL foaming capacity and 95% foam stability).

Fig. 3. Foaming Capacities (FC) and Foam Stabilities (FS) of Protein in Pennycress Seed Meal and Press Cakes



Emulsification Properties. Emulsifying activities of the pennycress seed and press cake proteins were excellent (Fig. 4). Seed and cold-press cake proteins were better emulsifiers than the cooked-pressed cake protein. Emulsions formed were also fairly stable.

Fig. 4. Emulsifying Activity Indices (EAI) and Emulsion Stability Indices (ESI) of Protein in Pennycress Seed Meal and Press Cakes



CONCLUSIONS

- Pennycress seed meal and press cakes contained substantial protein (>30% [db]), with dominant protein classes being water- and NaCl-soluble.
- Heat applied during cooking had detrimental effects on the protein, specifically on the water- and NaCl-soluble fractions.
- Despite generally poor solubility, pennycress seed protein and those in press cakes showed excellent emulsification and, most notably, foaming properties.
- These results showed that protein in pennycress seed and press cakes have functional properties that may be desirable for food and nonfood applications.

References

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