

Enhancing the Nutritional Value of Canola (*Brassica napus*) Meal Using a Submerged Fungal Incubation Process

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Abstract

The aim of this study was to determine the optimal fungal culture to increase the nutritional value of canola meal so it could be used at higher feed inclusion rates, and for a broad range of monogastrics, including fish. Submerged incubation conditions were used to evaluate the performance of seven fungal cultures in hexane extracted (HE) and cold pressed (CP) canola meal. *Aureobasidium pullulans* (Y-2311-1), *Fusarium venenatum* and *Trichoderma reesei* resulted in the greatest improvements in protein levels in HE canola meal, at 21.0, 23.8, and 34.8 %, respectively. These fungi reduced total glucosinolates (GLS) content to 2.7, 7.4, and 4.9 $\mu\text{M}\cdot\text{g}^{-1}$, respectively, while residual sugar levels ranged from 0.8 to 1.6 % (w/w). In trials with CP canola meal, the same three fungi increased protein levels by 24.6, 35.2, and 37.3 %, and final GLS levels to 6.5, 4.0, and 4.7 $\mu\text{M}\cdot\text{g}^{-1}$, respectively. Additionally, residual sugar levels were reduced to 0.3-1.0 % (w/w).

Keywords: canola, fungal incubation, glucosinolates, rapeseed, submerged incubation

1. Introduction

Canola (*Brassica napus*) is grown widely in Canada and the northern United States, and it is the second most abundant source of edible oil in the world (Aider & Barbana, 2011). Canola meal is also the second most abundant protein source for livestock feed, trailing soybean meal (Newkirk, 2009). The abundance and lower price of canola meal have driven interest in replacing soybean meal in ruminant and monogastric feeds (Lomascolo, Uzan-Boukhris, Sigoillot, & Fine, 2012). On a cost per Kg of protein basis, canola protein is typically valued at 80-85 % the value of soybean meal because it contains less gross energy, less protein, and over three times as much fiber. Canola also contains glucosinolates (GLS) that can have anti-nutritional effects on livestock. However, due to its lower cost it may be an economical protein source for animals that do not have high energy or lysine requirements (Bell, 1993).

The presence of GLS in canola meal limits inclusion levels in livestock diets, as they can be toxic when consumed at high levels, dependent on livestock species (Tripathi & Mishra, 2007). GLS and the enzyme myrosinase are compartmentally stored separately in *Brassica* spp. (Rask et al., 2000). Upon mechanical disruption or other stresses on plant tissues, myrosinase cleaves glucose from GLS, which produces toxic compounds such as nitriles, thiocyanates, and isothiocyanates. This self-defense mechanism evolved to reduce animal and insect browsing of the plant (Halkier & Gershenzon, 2006). When consumed, these toxic breakdown products can cause deleterious effects on the thyroid, and ultimately cause goiters from iodine deficiency (Burel et al., 2001). For this reason, canola was bred to contain lower levels of GLS and erucic acid (Newkirk, 2009). However, feed inclusion rates are still limited to 30 %, approximately, and this reduces the value of canola meal

(Newkirk, 2009).

Canadian based MCN Bioproducts Inc. (Saskatoon, SK, Canada) patented a process to fractionate high value protein concentrates from solvent and non-solvent expelled canola meal (Newkirk, Maenz, & Classen, 2006; Newkirk, Maenz, & Classen, 2009). These protein concentrates contained greater than 60 % protein, no detectable phytic acid, and less than 5 $\mu\text{M}\cdot\text{g}^{-1}$ of total GLS. However, this process utilizes multiple separation steps, which can be expensive and result in a relatively low protein yield in the primary marketed fraction. Bunge licensed this technology in 2012 (All About Food, 2012).

In contrast to mechanical separation to isolate protein, the metabolic diversity of fungi may be exploited to convert canola carbohydrates into protein-rich, single celled protein, and thereby produce a less expensive canola protein concentrate. In addition, fungal bioprocessing has been shown to significantly reduce GLS levels (Croft, Berhow, Karki, Muthukumarappan, & Gibbons, 2015). We hypothesized that this process would generate a more digestible product with enhanced nutritional value to a range of aquaculture and other livestock species. Fungi selected for initial evaluation included *Aureobasidium pullulans*, *Trichoderma reesei*, *Fusarium venenatum*, *Pichia kudriavzevii*, and *Mucor circinelloides*. Several of these fungi are known to produce cellulose degrading enzymes (Wiebe, 2002; Olempska-Beer, Merker, Ditto, & DiNovi, 2006; Seiboth, Ivanova, & Seidl-Seiboth, 2011; Prajapati, Jani, & Khanda, 2013; Ratledge, 2013). Studies have shown that *F. venenatum* is capable of producing mycotoxins, however their production can be avoided by controlling fermentation conditions (Wiebe, 2002). Both hexane extracted (HE) and cold pressed (CP) canola meals were evaluated with a submerged incubation process, which allowed for better activity of cellulolytic enzymes.

2. Material and Methods

2.1 Feedstocks and Preparation

The HE canola meal was obtained from North Dakota State University (Fargo, ND, USA), while CP canola meal was obtained from Agrisoma Biosciences (Ottawa, Ontario, Canada). Both HE and CP meals were milled through a 2 mm screen via FitzMill model # S-DAS06 knife mill (Elmhurst, IL, USA) prior to use, and were stored at room temperature in sealed bucket throughout the duration of experimentation. Dry weight (dw) analysis was conducted by drying 5 g of canola meal at 80 degrees Celsius ($^{\circ}\text{C}$) in a drying oven for at least 48 hours (h).

Cultures, Maintenance, and Inoculum Preparation

A. pullulans (NRRL-58522), *A. pullulans* (NRRL-42023), *A. pullulans* (NRRL-Y-2311-1), *T. reesei* (NRRL-3653), and *F. venenatum* (NRRL-26139) were obtained from the National Center for Agricultural Utilization Research (Peoria, IL, USA). *P. kudriavzevii* and *M. circinelloides* were isolated as contaminants from prior trials, and were identified by ARS-USDA (Peoria, IL, USA) using 15 s RNA analysis (O'Donnell, 2000). Short-term maintenance cultures were stored on Potato Dextrose Agar plates and slants at 4 $^{\circ}\text{C}$. Inocula for all experiments was prepared by transferring isolated colonies or a square section of agar growth (filamentous fungi) into glucose yeast extract (GYE) medium consisting of 5 % glucose and 0.5 % of yeast extract. The pH for *Aureobasidium*, *Pichia*, and *Mucor* cultures was adjusted to 3.0 ± 0.1 with 5 M sulfuric acid, while a pH of 5.0 to 5.5 was used for *T. reesei* and *F. venenatum*. GYE flasks consisted of 100 milliliter (mL) working volume in 250 mL Erlenmeyer flasks, covered with a foam plug and aluminum foil. Cultures were incubated for 72 h at 30 $^{\circ}\text{C}$ in a New Brunswick Scientific Excella E24 rotary shaker (Hauppauge, NY, USA) at 150 min⁻¹.

2.2 Experimental Procedures

Submerged trials were conducted in 1 L Erlenmeyer flasks with a working volume of 500 mL at a 10 % solid loading rate (SLR) dry weight canola meal. Flasks were covered with foam plugs and aluminum foil. For trials to be subjected to an initial saccharification step, 5 M sulfuric acid was used to adjust the initial pH to 5.0 ± 0.1 (this is the optimal pH level for the commercial cellulase and hemicellulase enzymes used). For trials lacking the saccharification step, the pH was adjusted to the levels indicated previously for specific microbes. Flasks were then autoclaved at 121 $^{\circ}\text{C}$ for 20 min. For saccharification trials, 0.052 mL CTec2 and 0.138 mL HTec2 (Novozymes, Franklinton, NC, USA) were added, and flasks were incubated at 50 $^{\circ}\text{C}$ and 150 min⁻¹ for 24 h. Following saccharification, the pH was adjusted, when necessary, for the specific microbes, and the slurry was cooled to 30 $^{\circ}\text{C}$. Saccharification and non-saccharification trials were inoculated with 5 mL of a 72 h culture of the appropriate organism and incubated at 30 $^{\circ}\text{C}$ at 150 min⁻¹ during 168 h. Daily samples of 50 mL were collected and used to monitor pH, cell counts, carbohydrates, protein, fiber, and GLS as described later. Daily samples and remaining slurry at the end of incubation was dried for 2 days at 80 $^{\circ}\text{C}$ using a Fisher Scientific Isotemp oven (Waltham, MA, USA).

2.3 Analytical Methods

2.3.1 Total Protein

The pH of each sample was measured in an Oakton 110 series pH meter (Vernon Hills, IL, USA). Forty-five mL of each sample were dried for 2 days at 80 °C. Approximately 0.5 g of each sample was used for protein analysis in duplicate. Protein was quantified using a LECO model FP528 (St. Joseph, MI, USA) to combust the sample and to measure the total nitrogen gas content in the sample (AOAC Method 990.03). Protein percentage was then calculated from the nitrogen content of the sample using a conversion factor of 6.25. An additional 0.25 g of sample was dried at 80 °C for 48 h to determine the dry matter of protein samples.

2.3.2 Residual Sugars

High Performance Liquid Chromatography (HPLC) was used to measure residual sugars using 5 mL of sample supernatant. Samples were firstly boiled for 10 min to ensure the fungal culture and/or saccharification enzymes were inactivated. Samples were then centrifuged at 10,000 min⁻¹ for 10 min, and the supernatant was poured into 2 mL microcentrifuge tubes and frozen overnight. The supernatant was then thawed and re-centrifuged at 10,000 min⁻¹ for 10 min to remove any precipitants. The final supernatant was then filtered through a 0.2 micrometer (µm) filter and into a HPLC vial and frozen until analysis. A Waters size-exclusion chromatography column (SugarPak column I10 µm, 6.5 mm X 300 mm with pre-column module, Waters Corporation, Milford, MA, USA) and a HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with refractive index detector (Model G1362A) were used to measure the sugars. The sugars were eluted using a de-ionized water as mobile phase at flow rate of 0.5 mL.min⁻¹ and column temperature of 80 °C. Sugars to be quantified included arabinose, galactose, glucose, raffinose, stachyose, and sucrose. All sugar standards were purchase from Sigma-Aldrich (St. Louis, MO, USA) while all standards contained a purity of 99.9 %. The sugar standards were prepared using several concentrations and a calibration curve was constructed using concentration versus HPLC area previously established by Karunanithy, Karuppuchamy, Muthukumarappan, & Gibbons (2012).

2.3.3 Glucosinolates

Approximately 1.5 g of dried 0 h and 168h sample were used for GLS analysis. Individual GLS were confirmed to be present by quadrupole time-of-flight liquid chromatography-mass spectrometry and quantified using reverse phase HPLC (Berhow et al., 2013). For GLS quantitation, a modification of a HPLC method, developed by Betz and Fox (1994), was used. The extract was run on a Shimadzu (Columbia, MD) HPLC System (two LC 20AD pumps; SIL 20A autoinjector; DGU 20As degasser; SPD-20A UV-VIS detector; and a CBM-20A communication BUS module) running under the Shimadzu LC solutions Version 1.25 software. The column was a C₁₈ Inertsil reverse phase column (250 mm X 4.6 mm; RP C-18, ODS-3, 5µ; with a Metaguard guard column; Varian, Torrance, CA). The glucosinolates were detected by monitoring at 237 nm. The initial mobile phase conditions were 12 % methanol / 88 % aqueous 0.005 M tetrabutylammonium bisulfate (TBS) at a flow rate of 1 mL.min⁻¹. After injection of 15 µL of sample, the initial conditions were held for 2 min, and then up to 35 % methanol over another 20 min, then to 50 % methanol over another 20 min. then up to 100 % methanol over another 10 min.

2.3.4 Fiber

Fiber analysis was completed as Neutral Detergent Fiber (NDF) and Acid Detergent fiber (ADF). NDF is a method commonly used for animal feed analysis to determine the amount of lignin, hemicellulose and cellulose, while ADF represents the least digestible fiber fraction of animal feed including lignin, cellulose, silica but not hemicellulose. NDF and ADF analysis were completed by Midwest Laboratories (Omaha, NE, USA) using ANKOM Technology (Macedon, NY, USA) filter bag methods. Approximately 3 g of dried material was submitted in triplicate for each treatment combination.

3. Results and Discussion

Seven fungal strains were grown on HE vs CP canola meal using a submerged incubation process. Submerged incubation has been defined as processing in the presence of excess water, and has been a proven large-scale process due to easier material handling and process control (Singhania, Sukumaran, Patel, Larroche, & Pandey, 2010). In contrast to solid-state incubation completed in previous work (Croat, Berhow, Karki, Muthukumarappan, & Gibbons, 2016a), submerged incubation has the advantage of being a more homogenous mixture while allowing improved streamlining and standardization of processing (Chicatto, Costa, Nunes, Helm, & Tavares, 2014). The fungi were tested both on raw (non-saccharified) and saccharified meal slurries using commercial cellulases to enhance fiber breakdown. These trials were done in shaker flasks, where mixing and mass transfer were the limiting factors. However, these non-optimized trials were meant to quickly down-select

the best microbe for each type of canola meal. Other investigators have previously used a similar submerged incubation process to quickly assess phytase activity of various strains of bacteria, yeasts and fungi when grown on canola and oilseed meals (Nair & Duvnjak, 1991).

3.1 Total Protein

Figures 1 and 2 present the maximum protein levels in HE and CP canola meals, respectively, for raw meal and un-inoculated controls versus the various fungi, both under non-saccharified and saccharified conditions. As expected, protein levels for the un-inoculated controls were similar to the raw meals. In HE meal, protein levels increased from 36.1 % in the raw meal to 39.0-48.7 % after the fungal conversion process (relative improvements of 8.0-34.9 %) (Fig 1). The *M. circinelloides* trial was the only one in which an enzymatic hydrolysis step prior to inoculation proved beneficial. In the case of *T. reesei*, the non-saccharified trial actually resulted in higher protein titers. We had anticipated that saccharification would have a significant positive effect on fiber hydrolysis, and subsequently protein levels. It could be that canola fibers require pretreatment to increase susceptibility to enzymatic hydrolysis (Gattinger, 1990; Yaun, 2014). Proceeding work investigated various pretreatment methods to make canola fibers more susceptible to hydrolysis by the fungal enzymes, thus releasing more sugar for conversion into single celled protein (Croat, Berhow, Iten, Karki, Muthukumarappan, & Gibbons, 2016b). This proceeding work observed pretreatments including extrusion, hot water cook, dilute acid, and dilute alkali compared to non-pretreated canola meal.

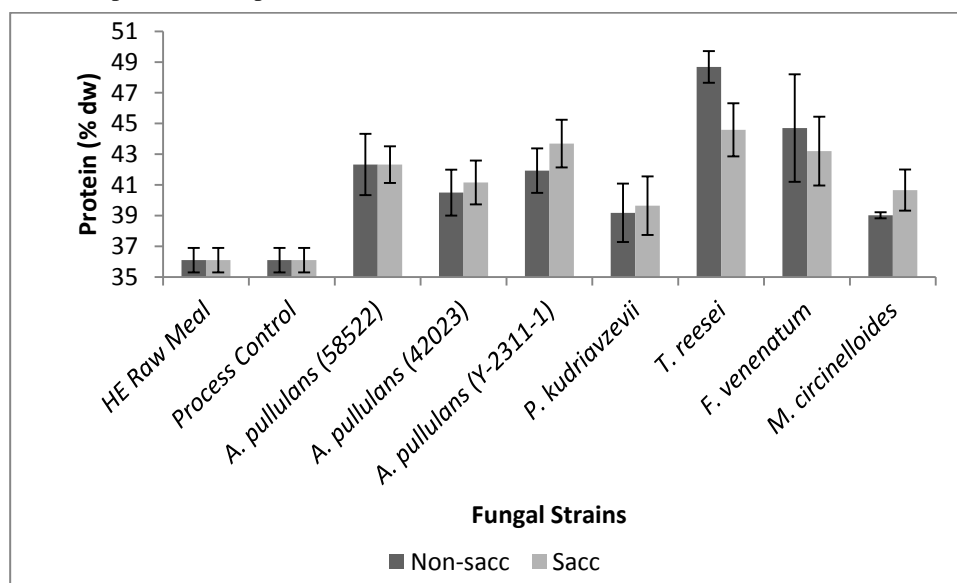


Figure 1. Maximal protein levels \pm SD of HE canola meal following submerged fungal incubation

In the CP canola meal (Fig 2) the protein level in the un-inoculated control was 38.6 %, and rose from 40.9 to 53.0 % after microbial conversion, representing relative improvements of 6.0-37.3 %. CP canola meal was about 3 % higher in protein than HE meal and following incubation, protein levels were ~2-8 % higher in CP canola meal trials compared to HE meal for each pair of fungi. HE is a more effective method of removing oil from canola seed, however this process applies significantly higher levels of heat, which may denature or degrade some protein (Spragg & Mailer, 2007). We observed that the enzymatic hydrolysis step prior to inoculation did not significantly affect protein levels for all the fungi tested. Thus for un-pretreated canola meal, there was no benefit to adding cellulolytic enzymes.

T. reesei achieved the highest protein levels for both substrates, while *P. kudriavzevii* exhibited the lowest protein enhancement. *T. reesei* is known to produce many hydrolytic enzymes (Li et al., 2013), and it was expected to provide the greatest conversion of fiber and oligosaccharides into cell mass. As a single-celled yeast, *P. kudriavzevii* does not produce cellulase enzymes and it was therefore anticipated to result the lowest protein improvement. The final protein levels for all other fungal strains were relatively similar, at 40-45 % in HE canola meal and 43-52 % protein in cold pressed canola meal.

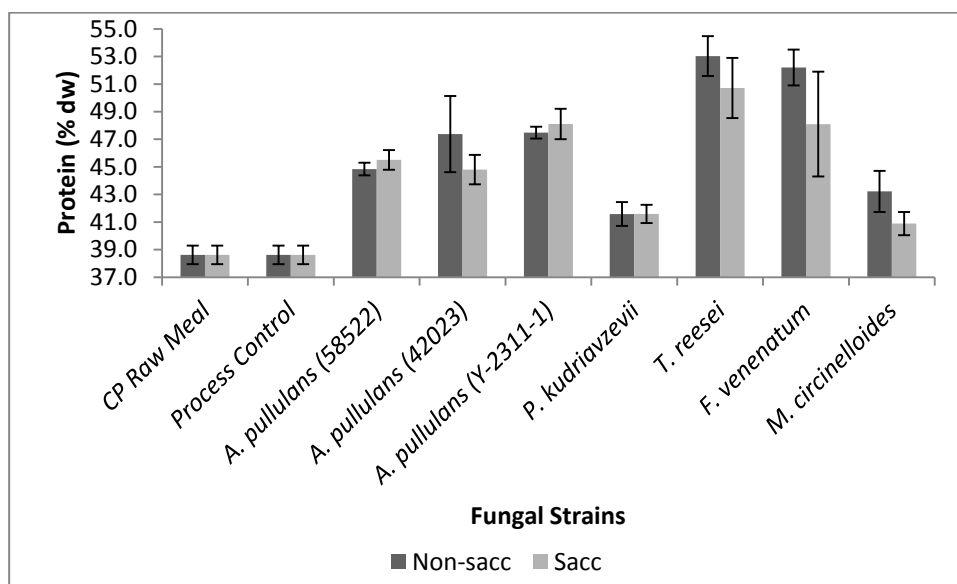


Figure 2. Maximal protein levels \pm SD of CP canola meal following submerged fungal incubation

3.2 Residual Sugars

Arabinose, galactose, glucose, raffinose, stachyose, and sucrose were measured throughout incubation via HPLC. For simplicity, the final levels of these sugars were combined and are presented as residual sugars in Figures 3 and 4 for HE and CP canola meal, respectively. The total residual sugar concentrations decreased slightly (2.7-5.5 %) from the raw meals compared to the process controls. Nyombaire, Siddiq, and Dolan (2007) found that a pre-soaking and 80 °C of cooking temperature were sufficient to hydrolyze oligosaccharides such as raffinose and stachyose in red kidney beans. Autoclaving the 10 % SLR canola slurries may have achieved a similar effect, thereby reducing the raffinose and stachyose concentrations.

Between 37.0-94.6 % of sugars present in non-saccharified HE meal (Fig. 3) were used by the fungi during incubation, resulting in residual sugar levels of 0.8-9.4 %. Similarly, 39.0-88.6 % of sugars present in saccharified HE meal was utilized by the fungi, resulting in residual sugar levels of 1.7-9.1 %. *T. reesei* exhibited the lowest residual sugar levels on both non-saccharified and saccharified HE meals, while *M. circinelloides* and *P. kudriavzevii* had the highest final levels in non-saccharified and saccharified trials, respectively. *M. circinelloides* did show a benefit from saccharification, showing a significant drop in residual sugars from 9.4 to 2.7 % w/w when compared to non-saccharification.

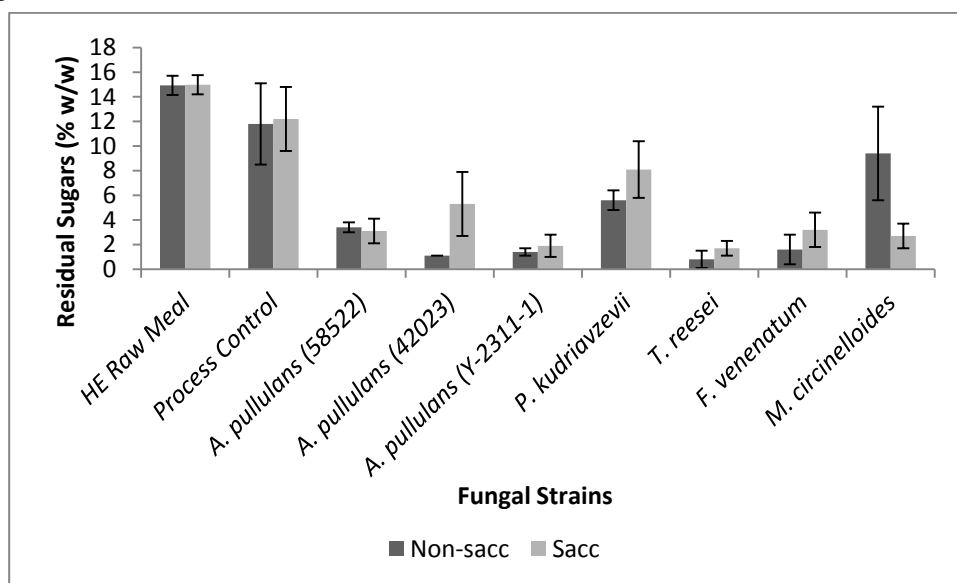


Figure 3. Residual sugar levels \pm SD of HE canola meal following submerged fungal incubation

In non-saccharified CP meal (Fig. 4) between 61.0-98.1% of sugars present were metabolized by the fungi during incubation, decreasing residual sugar levels to 0.3-6.3%. Similarly, 40.0-95.0% of sugars present in saccharified CP meal were metabolized by the fungi during incubation, decreasing residual sugar levels to 0.8-9.7%. *F. venenatum* and *T. reesei* exhibited the lowest residual sugar levels on both non-saccharified and saccharified CP meal, while *A. pullulans* (NRRL-42023) and *P. kudriavzevii* had the highest final levels in non-saccharified and saccharified material, respectively. Saccharification significantly reduced residual sugars in trials with *M. circinelloides* and *A. pullulans* (NRRL-42023) when compared to non-saccharification trials.

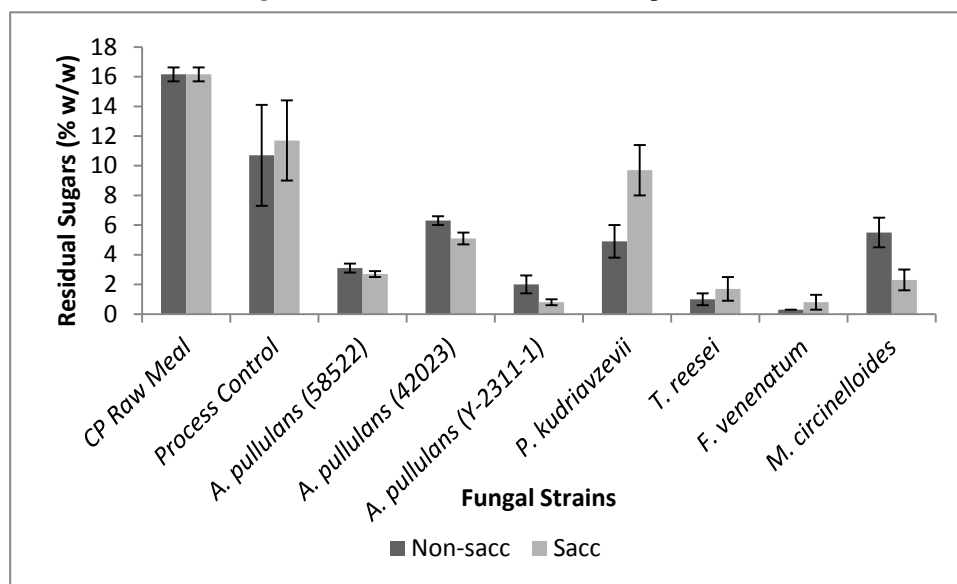


Figure 4. Residual sugar levels \pm SD of CP canola meal following submerged fungal incubation

3.3 Glucosinolates

Figures 5 and 6 show GLS levels for the HE and CP canola meal trials, respectively. GLS levels were reduced from $42.8 \mu\text{M.g}^{-1}$ in raw HE meal to $8.7 \mu\text{M.g}^{-1}$ (non-saccharified) and $18.3 \mu\text{M.g}^{-1}$ (saccharified) in the un-inoculated process controls. This represents 79.6 and 57.2 % reductions, respectively, and was presumed due to the conversion of some of the GLS into volatile breakdown products (Halkier & Gershenzon, 2006). Newkirk, Classen, Scott, and Edney (2003) also noted that high processing heat can be used to remove volatile anti-nutritional factors; however this can also denature proteins. Submerged microbial conversion further reduced GLS content to 1.0 - $14.4 \mu\text{M.g}^{-1}$, representing a total reduction of 66.5-97.8 %.

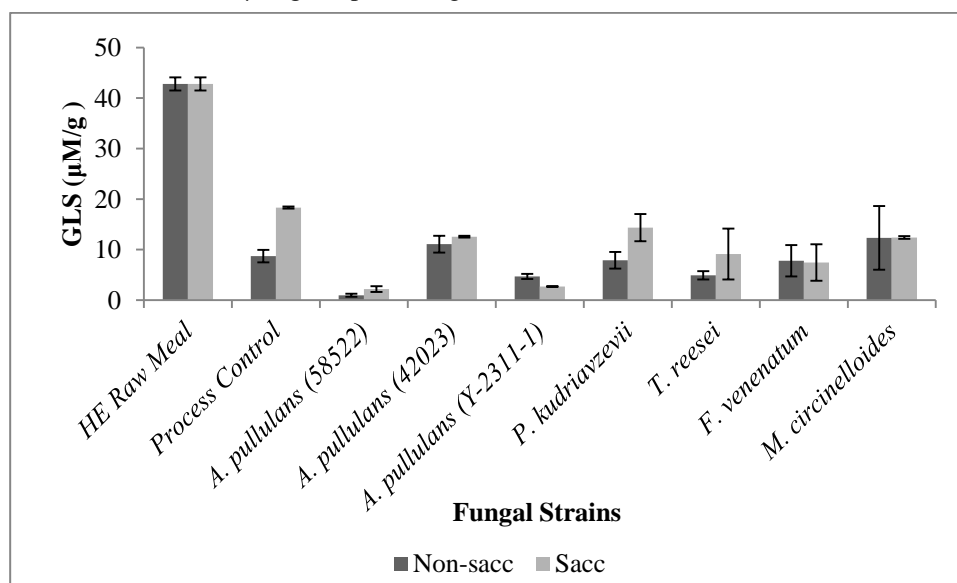


Figure 5. Reduction of total GLS \pm SD following sterilization and submerged fungal incubation in HE canola meal

GLS levels in raw CP meal ($60.6 \mu\text{M.g}^{-1}$) were higher than in HE meal ($42.8 \mu\text{M.g}^{-1}$) since the former does not include the high temperature step to remove the extraction solvent (hexane), which can eliminate GLS. Treatment of the CP meal with the autoclaving and drying steps in the process control reduced GLS levels to 18.6 and $26.2 \mu\text{M.g}^{-1}$, respectively in non-saccharified and saccharified trials (reduction of 69.4 and 56.8 %, respectively). Again, submerged microbial conversion further reduced GLS content to $0.7\text{-}23.7 \mu\text{M.g}^{-1}$ (total reduction of 60.8-98.9 %).

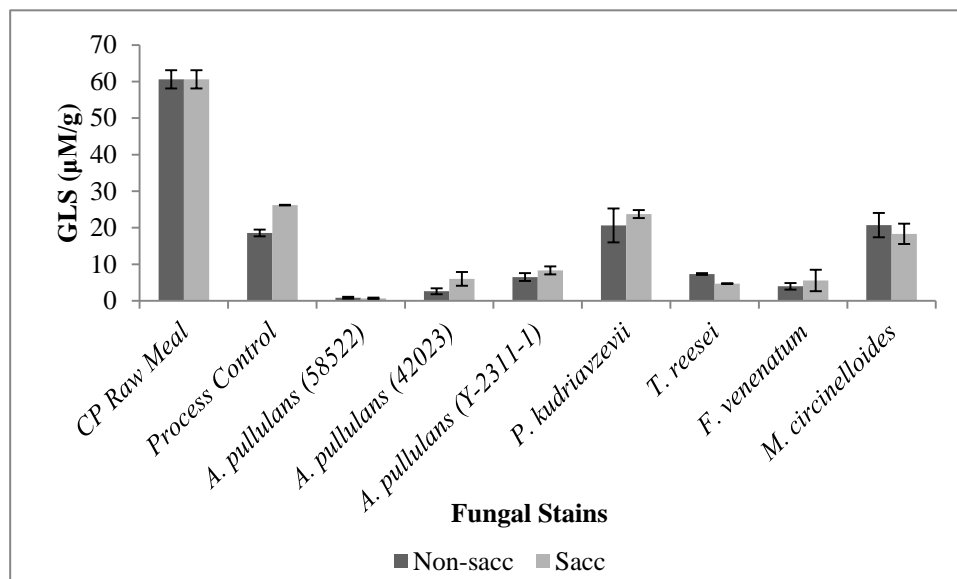


Figure 6. Reduction of total GLS \pm SD following sterilization and submerged fungal incubation in CP canola meal

Overall, *A. pullulans* (NRRL-58522) caused the greatest reduction in GLS levels in both HE and CP canola meals (ranging from 94.5-98.9%), likely due to its robust capability for producing extracellular enzymes (Kudanga & Mwenje, 2005). *A. pullulans* (NRRL-Y-2311-1) was also very effective in reducing GLS concentrations (ranging from 86.3-93.7%), followed by *F. venenatum* (81.8-93.5%) and *T. reesei* (78.7-92.2%). Previous studies have shown that various microbes are able to degrade GLS and metabolize the resulting glucose and sulfur moieties. For example, Vig & Walia (2001) observed that *Rhizopus oligosporus* reduced GLS and their byproducts during fungal incubation of *Brassica napus* meal. Similarly, Rakariyatham & Sakorn (2002) reported the complete degradation of GLS after 60-96 h using solid-state fermentation of *Brassica juncea* with *Aspergillus* sp. In the work reported herein, *P. kudriavzevii* and *M. circincelloides* resulted in the least reduction in GLS, as expected due to minimal production of extracellular hydrolytic enzymes when compared to the other fungi tested.

3.4 Fiber

Table 1 provides the ADF and NDF fiber levels of raw, process control, and treated canola meals. In general, most fiber levels were statistically similar to the raw meal, indicating that the conversion process had minimal effects on fiber levels. The only trial to show a statistically significant reduction in ADF in HE meal was *P. kudriavzevii*, while trials with *A. pullulans* (Y-2311-1), *P. kudriavzevii*, *T. reesei*, *F. venenatum*, and *M. circincelloides* all statistically reduced ADF and/or NDF fiber levels in CP canola meal (Table 1). Thus the cellulase producing fungi were effective in hydrolyzing fiber in CP canola meal, however did not show similar results in HE canola meal. A possible explanation for the reduced fiber degradation in HE canola meal is that the heating steps of the hexane extraction process may have reduced the susceptibility of the fibers to subsequent enzymatic hydrolysis. Also, the enzyme cocktail used in the saccharification trials were not optimized for canola fiber, and this may provide a future opportunity to enhance fiber degradation.

In some cases the conversion process actually resulted in a concentration of fibers, caused by the removal of sugars and GLS. Trials with *A. pullulans* (58522), *A. pullulans* (Y-2311-1), *F. venenatum*, and *M. circincelloides* all increased fiber levels in HE canola meal, while *A. pullulans* (58522) and *A. pullulans* (42023) treatments both increased ADF and/or NDF fiber levels in CP canola meal (Table 1).

We have previously shown that feedstock pretreatment increases the susceptibility of fibers to hydrolysis (Karki, Muthukumarappan, & Gibbons, 2013), and that optimizing the fungal incubation conditions will also enhance

cellulase production and activity. This will be evaluated in future studies using extrusion, hot cook, dilute acid, and dilute alkali pretreatments. The resulting sugars would then be available for conversion into additional cell mass and protein

Table 1. Fiber reduction of non-saccharified and saccharified canola meal during submerged fungal incubation

	Hexane Extracted				Cold Pressed			
	Non-Saccharified		Saccharified		Non-Saccharified		Saccharified	
Fungal Culture	ADF (%)	NDF (%)	ADF (%)	NDF (%)	ADF (%)	NDF (%)	ADF (%)	NDF (%)
Raw Meal	19.9±0.2	23.1±0.3	19.9±0.2	23.1±0.3	11.5±0.5	15.0±0.3	11.5±0.5	15.0±0.3
Process Control	18.7±0.3	22.0±0.8	23.0±1.1	29.0±1.8	9.5±0.6	12.4±0.8	14.8±1.4	16.1±1.6
<i>A. pullulans</i> (NRRL-58522)	22.0±1.6 ^b	29.1±0.8 ^b	20.6±2.4	25.2±4.6	12.1±1.0	16.8±0.7 ^b	11.6±1.1	15.9±0.6
<i>A. pullulans</i> (NRRL-42023)	20.4±1.5	24.3±1.3	19.4±2.2	22.6±0.5	12.4±0.4	16.9±0.4 ^b	11.2±1.1	15.1±0.3
<i>A. pullulans</i> (NRRL-Y-2311-1)	22.3±0.9 ^b	24.5±0.7 ^b	21.2±1.4	24.0±0.9	13.6±2.1	14.8±2.4	11.0±0.9	12.6±1.0 ^a
<i>P. kudriavzevii</i>	19.7±1.6	23.1±1.9	18.6±0.3 ^a	22.7±1.9	11.0±0.4	13.5±0.3 ^a	10.4±0.4 ^a	12.4±0.5 ^a
<i>T. reesei</i> (NRRL-3653)	19.9±3.1	22.5±4.0	19.8±0.8	26.4±3.3	7.6±0.8 ^a	10.1±0.6 ^a	8.1±1.2 ^a	10.8±2.0 ^a
<i>F. venenatum</i> (NRRL-26139)	21.3±2.4	26.7±2.6 ^b	20.8±2.2	26.9±0.7 ^b	7.6±0.9 ^a	10.7±1.2 ^a	10.7±2.5	12.9±3.0
<i>M. circinelloides</i>	21.0±0.5 ^b	25.9±1.2	19.6±1.2	22.6±1.0	10.2±0.9	12.8±0.9 ^a	10.8±0.6	15.6±1.3

^aIndicates fiber level was statistically lower than raw meal.

^bIndicates fiber level was statistically higher than raw meal.

4. Conclusions

Submerged incubation with various fungal strains improved the nutritional content of canola meal. *T. reesei* (NRRL-3653), *F. venenatum* (NRRL-26139), and *A. pullulans* (Y-2311-1) resulted in the greatest improvement in protein content in HE canola meal (34.8, 23.8, and 21.0 %), respectively, while reducing total GLS and residual sugar content by 82.6-93.7 % and 89.3-94.6 %. In trials with CP canola meal, the same three fungi increased protein levels to the greatest extent (37.3, 35.2, and 24.6 %), respectively, while reducing total GLS and residual sugar content by 89.3-93.5 % and 93.8-98.1 %.

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