Selection of Fungus Stains for Canola Meal Fermentation

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Abstract

Canola seed is now a popular alternative to soybeans for oil extraction. The canola oil industry has widely spread into Canada, Europe and Asia. Locally in Minnesota, canola seeds are processed to the scale of 100,000 bushel/year. One of the byproducts of canola oil refining is the canola meal, which can be processed further and sold as animal feed. There has been interest in replacing soybean meal with canola meal due to its overall higher protein content and improvements to quality in animal products and lower cost of raw materials compared to soybean meal. As different types of fungal have been discussed in related journals, three specific fungal strains are of concern in this project. Trichoderma reesei, capitalized as TR, is mainly tested to find its role in converting cellulose in canola meal into protein. HPLC test is done to compare the amount of amino acid and other contents before and after the fermentation. The project has proved that the fermentation is beneficial to the feed to increase the protein content so as to make the feed more nutritional, and the fungal chain which work best in the given period with the same amount of substrate is also found for different objectives.

Introduction

Canola meal is a potent nutritional meal for cattle and swine. However, as the cellulose content shows high in canola meal compared to the more popular soybean meal, there is emerging necessity that further processing on canola meal. Fermentation is an excellent way to increase the protein content in the feed, and the choice of fungal chain is to be made among Trichoderma reesei (TR), Mucor indicus (MI), and Rizopus oryzae (RO). The HPLC test determines the structural carbohydrate and amino acid content before and after fermentation. Each testing and treatment would be done to both canola meal and soybean meal with various fungal chains for comparison.

It was hypothesized that the TR chain would show the best performance on improving the protein content of the meal by hydrolyzing cellulose and hemicellulose in the meal and then convert the sugar to amino acids. As the nitrogen content in the meal is limiting the conversion, 1.0% urea solution is added in trials to test its effect on amino acid growth.

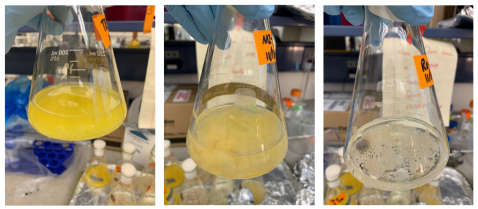


Figure. The fully developed TR, MI and RO mycelia liquid culture used for fermentation

Methodology

Trichoderma reesei (TR), Mucor indicus (MI) and Rizopus oryzae (RO) were maintained in potato dextrose agar (PDA) medium for 7 days at 30 degree C for better development of spores of the fungi. Then, five pieces of PDA medium along with the mycelia (0.5 square meter area for each) were cut from the plate and transferred to 50mL of potato dextrose broth (PDB) medium (freshly sterilized at 121 degree C for 20 minutes) placed in 250mL Erlenmeyer flask for each. The liquid sub-cultures were then again placed in the incubator for 4 days at 25 degree C for further culture shaking at 150 rpm.

For the fermentation step, 5mL of the fully developed mycelia was transferred to each Erlenmeyer flask as fermentation container. Groups of Control (no fungal chain), TR, MI and RO were settled to make comparison, and each had no-urea group and urea group. Three parallels were made for each case. In each trial, 11.3g soybean meal (SM) or 10.8g canola meal (CM) is added to ensure 10.0g dry solid, and 70% moisture was maintained by adding deionized (DI) water to the culture. The treated cultures were then fermented for 8 days and dried at 60 degree C for 48 hours after fermentation. The dried product was grounded with a portable coffee grinder for nutrition value testing.

Each grounded sample was hydrolyzed with 0.6M HCl for amino acid analysis. For structural carbohydrate analysis, 100 mg of each grounded dried sample was hydrolyzed with 72% wt. sulfuric acid, and treated with water bath later, and then vacuum filtrated to get the spectrophotometry result. By neutralization and further filtration through 0.25μm membrane, the HPLC-standard liquid with pH value close to 7.0 was prepared for HPLC test, and the peaks would give detailed analysis on composition and amount of different carbohydrates.

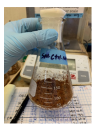
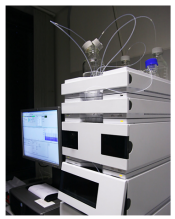
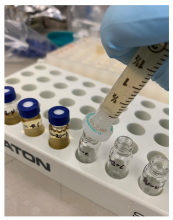


Figure. The Ctrl, the growing fermenting meal, and the dried meal

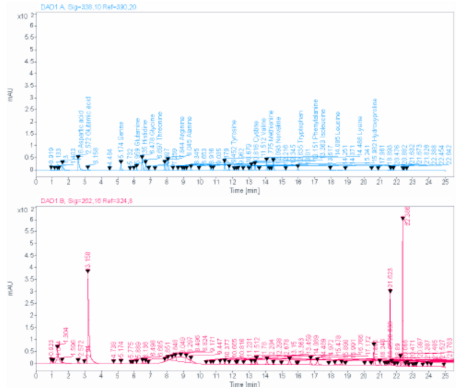
Results

'High Performance Liquid Chromatography (HPLC) is a form of column chromatography that pumps a sample mixture or analyte in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase).'

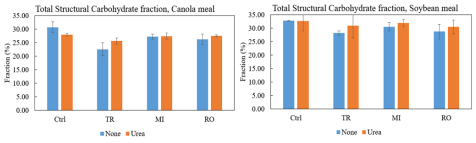




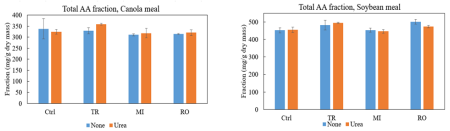
By doing the HPLC test, the peaks of structural carbohydrates and amino acids have shown how effectively the fungal chains converted the original content of the meals to valuable amino acids for the animals. The remainders were also of concern.



In the structural carbohydrate part, it was found that for both SM and CM the addition of fungal strains would decrease the amount of structural carbohydrate significantly. The fungal chain TR has shown best performance in reducing the structural carbohydrate, which is expected. The TR proved its ability of decomposing the cellulose in the animal feed as well as converting the sugar to protein, which is exactly the expectation to meet in improving the nutritional value of the feed.



Conclusion



It was shown that the fungal chain Trichoderma reesei was showing the best performance in improving the amino acid content; furthermore, the addition of urea solution helped develop the backbone of the amino acids, and a significant advantage of amino acid growth was shown in the CM-TR treatment case. However, the urea addition didn't show as much help in the MI- and RO-treatment cases and ever reduced the growth of amino acid in the soybean meal case. Thus, it could be concluded that urea is especially a valuable addition in the Trichoderma reesei treatment fermentation of canola meal.

CM	Thr		Arg		Met		Lys	
Key AA content (mg)	None	Urea	None	Urea	None	Urea	None	Urea
Ctrl	16.37	15.71	22.38	22.59	6.18	5.97	18.11	13.22
TR	18.01	18.73	17.91	21.05	4.84	7.19	16.60	15.13
MI	15.70	16.20	20.76	22.75	4.98	5.44	16.92	13.73
RO	15.14	15.70	20.89	22.20	4.79	5.14	15.84	13.66

Reflections

The well-designed experiments helped me much in achieving my goal of deciding the best fungal chain to make canola meal a more competitive animal feed in the global market. Though the tests on amino acids and structural carbohydrates have given information that there is still a long way to go to replace the soybean meal with canola meal, the fermentation experiment still proved that a good choice of the fungal chain would help much in improving the nutritional value of the canola meal.

The research further exercised me to step into the real career of a biochemistry-focused engineer with trained engineering designing knowledge and experience. As the core of my major is to think like a real engineer by solving problems with well-developed research, the UROP research is beneficial to my own understanding of the industry as well as the core of the bioproduct concept.

References

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