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# *EVALUATION OF MEANS OF REDUCING GLUCOSINOLATE IN CANOLA MEAL*

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***ABSTRACT***

***Introduction:*** *Canola oil is the healthiest oil. However, processing canola oil resulted in the by-product, canola meal. Canola meal is a comparable animal feed to soybean meal provided that the glucosinolate is removed.* ***Methods****: Soaking and heating was selected as the method of reducing glucosinolates in canola meal, as this technique is can be easily adapted. The total glucosinolates were determined based on the reaction with thymol and sulfuric acid. It was applicable to industry as it was fast and did not require myrosinase purification. The quantity of glucosinolates in untreated canola meal was 17.25 µmol/g (<30 µmol/g). The treatments consist of soaking and heating. The soaking times were 4,8 and 12 hours, followed by heating (using oven) for an hour at 40, 60 and 80°C. The treated canola meals showed reduction in glucosinolates.* ***Result:*** *There was a significant reduction in glucosinolates in treated meal compared to untreated. However, there was no significant difference in glucosinolates content between different soaking and heating treatments.* ***Conclusion****: Therefore, 4 hours soaking followed by heating for 1 hour at 40°C was considered satisfactory to reduce glucosinolates from canola meal.*

***Key words*** *: canola meal, soaking, heating, glucosinolate*

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# EVALUASI PENGURANGAN GLUKOSINOLAT DI AMPAS KANOLA

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**ABSTRAK**

**Pendahuluan**: Minyak kanola adalah minyak yang paling menyehatkan, namun hasil sampingan dari pembuatan minyak kanola menghasilkan ampas kanola (*canola meal*). *Canola meal* adalah makanan hewan yang sebanding dengan ampas kedelai bila glucosinolate-nya dihilangkan/direduksi. **Metode:** Perendaman dan pemanasan dipilih sebagai metode pereduksi glucosinolate dalam *canola meal*, karena teknik ini sangat mudah dilakukan. Total glucosinolate ditentukan berdasarkan reaksi dengan thymol dan asam sulfat. Hal ini dapat diaplikasikan di industri karena cepat dan tidak memerlukan purifikasi myrosinase. Kuantitas glucosinolate *canola meal* yang tidak diberi perlakuan adalah 17.25 µmol/g (<30 µmol/g). Perlakuan terdiri dari perendaman dilanjutkan pemanasan. Waktu perendaman adalah 4,8 dan 12 jam, diikuti oleh pemanasan (dengan oven) selama 1 jam pada temperatur 40, 60 dan 80°C. **Hasil:** *Canola meal* yang diberi perlakuan tereduksi berbeda secara bermakna kadar glucosinolate-nya dibandingkan yang tidak diberi perlakuan, namun, tidak ada perbedaan bermakna kandungan glucosinolate antara berbagai perlakuan yang berbeda. **Simpulan:** perendaman selama 4 jam yang diikuti pemanasan selama 1 jam pada 40°C dianggap cukup untuk mereduksi glucosinolate dari *canola meal*.

**Kata kunci :** *canola meal,* perendaman, pemanasan, *glukosinolate*

## INTRODUCTION

In 1979, the appellation of canola was adopted in Canada to refer all "double-low" rapeseed cultivars, specifically the cultivars that contain less than 2% erucic acid in their oil and less than 30 µmol/g of one or any combination of the four aliphatic glucosinolates (gluconapin, progoitrin, glucobrassicanapin, napoleiferin) in defatted meal. An example of double low cultivar is Tower, which is one of the main cultivar grown in Canada.

The production of double-low seeds from a *Brassica campestris* cultivar was achieved in a short time following the production of *Brassica napus* cultivars, "triple-low"cultivars, such as Candle were produced. These are called "triple-low" as they exhibit a low content in erucic acid, glucosinolate and fibre.[1] From an agronomical perspective, two varieties of canola can be distinguished, that are winter and spring canola varieties. In Australia, only spring varieties are sown.[2] The examples of the spring varieties are *Brassica napus* L., spp. *Oleifera* (non-GM canola), Sirena and Karoo (triazinetolerant)[3]; Roundup Ready ® and InVigor® (herbicide-tolerant).[4,5]

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Canola, is not native to Australia, and originated in either the Mediterranean area or Northern Europe. It was first grown commercially in Australia in 1969. Since 1970, canola or the doublelow cultivars has been bred extensively, including in Australia, due to its use. Canola is used to prepare the canola oil, and the canola meal is produced as a byproduct[2]. Canola meals contain about 38% protein. It has a well-balanced amino acid composition. Therefore, canola meal is a good animal feed. [6-10] On the other hand, the glucosinolate content limits the canola meal usage.

Glucosinolate and its metabolites produced by enzymatic breakdown have detrimental or toxic effects. The amino acid distribution is very complementary to soybean meal and the two meals are often included in the same ration. Feeding trials have shown that animals perform better when fed a mixture of the two meals than when fed either alone. In Canada, canola meals are recommended for up to 10-20 percent of the ration for chickens, turkeys, ducks, geese, pigs, dairy, and beef cattles.[11] In aquaculture, canola meal can be fed to Chinook salmon and red seabream.[12] Detoxification is the term used to describe the methods used for removing glucosinolates and/or their breakdown products. This includes chemical, microbiological, or physical treatments.

Examples of chemical treatments includes the gaseous ammonization, which removes up to 74% glucosinolate[13], and methanol-ammonia-water/hexane extraction system, which removes 72% of the phenolic compounds in canola meal.[7] Physical treatments includes heating, toasting for 30 minutes reduced the sinapine content by 17%[14] and extrusion[15], which can remove up to 67% of the glucosinolate content. The detoxification by chemical methods is expensive and has some other disadvantages including chemical changes. Therefore, an easy to be applied means of glucosinolate reduction could be conducted by physical treatment. In this research soaking and heating using low temperatures were being used.

## MATERIALS AND METHODS

### MATERIALS

Canola meal samples were obtained from Riverland Oilseed Processors Pty Ltd. in October 2004.

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## METHODS

### TREATMENT OF THE CANOLA MEAL

The canola meal samples (10 g) were soaked in water (ratio of meal:water 1:10, w/v) at room temperature (21 °C) for 4,8 or 12 hours, and then drained by using a vacuum filter. The samples were heated in oven at 40, 60 and 80°C for 1 hour depending on the treatment (table1). Each treatment was conducted in 3 replicates.

**Table 1. Treatment Performed**

| Treatments | Soaking Times (hours) | Temperature (°C) |
|---|---|---|
| 1 | 4 | 40 |
| 2 | 4 | 60 |
| 3 | 4 | 80 |
| 4 | 8 | 40 |
| 5 | 8 | 60 |
| 6 | 8 | 80 |
| 7 | 12 | 40 |
| 8 | 12 | 60 |
| 9 | 12 | 80 |
| 10 | Control | Control |

### DETERMINATION OF MOISTURE

The moisture content was measured by AOAC method[16] and calculated using the following formula :

$$MC\ (\%) = \frac{(W1-W2)}{(W1-W0)} \times 100$$

W0 = Weight of empty pre-dried aluminium dish

W1 = Weight of aluminium dish with canola meal before drying

W2 = Weight of aluminium dish with canola meal after drying

### DETERMINATION OF GLUCOSINOLATES

The glucosinolate content of canola meal was measured by the spectrophotometric method of DeClerq and Daun (1989).[17]

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**PREPARATION OF COLUMNS**

Sephadex A-25 (100 mg) was weighed and put into an ion exchanger column and allowed to swell with deionized water while being stirred to remove air bubbles. The procedure left water reservoir at the top of the column for rinsing and sample application. Prior to sample application, 5 mL of 0.5 N sodium hydroxide was passed through the columns, followed by water to remove excess sodium hydroxide. The eluate was monitored by pH meter (TPS LC 80A) to ensure the pH was neutral. Column was changed to acetate form by addition of 5 ml of 0.5 M pyridine acetate solution (930 ml deionized water + 30 ml glacial acetic acid + 40 ml pyridine) and then 10 ml water leaving a meniscus of water on top of the column for sample application.

**EXTRACTION OF GLUCOSINOLATES**

Non-treated canola meal 200 mg or treated canola meal 300 mg (to compensate for the loss due to soaking and heating) was weighed into a centrifuge tube and then placed in a waterbath at 95°C for 15 minutes. Four ml boiling water was added and the tube was fitted with cap and mixed quickly in a vortex mixer. The tube was kept in waterbath and heated at 95°C for three minutes. After cooling and centrifuging at 600x g for 5 minutes, the supernatant was transferred to a 10 ml graduated centrifuge tube containing 150 µl of a 0.5 M barium/lead acetate solution. The resulting pellet was extracted with an additional 4 ml of boiling water for three minutes at 95°C. After cooling and centrifuging, the supernatant from the second extraction was added to the first, and the total volume was adjusted to 10 ml. The total extract was centrifuged and it was refrigerated several days before testing.

**ISOLATION OF GLUCOSINOLATES**

An aliquot (3 ml) of the total extract was added to the prepared columns. Then the column was washed with 2x2 ml water, 2x2 ml 30% formic acid and 2x2 ml water, discarding the eluate each time. Glucosinolates were eluted with 2x 4.75 ml of 0.3 M potassium sulfate and adjusted to 10 ml).

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**MEASUREMENT OF GLUCOSINOLATES**

*Samples*

Aliquots of 1.0 ml were placed in clean borosilicate tubes and 7.0 ml of 80% sulfuric acid followed by 1 ml of 1% thymol in ethanol were added. Tubes were capped with the screw caps and thoroughly mixed and placed in a water bath at 100°C for 60 min, cooled under tap water and mixed.

*Blanks*

Blanks consisted of 1 ml of 0.3 M potassium sulfate, 7 ml of 80% sulfuric acid and 1 ml of 1% thymol solution (1% thymol in ethanol).

*Standards*

Standards were prepared with 1 ml of 0.3 µmol/ml sinigrin solution (0.0125 gram sinigrin in 100 ml deionized water), 7 ml of 80% sulfuric acid and 1.0 ml of 1% thymol solution (1% thymol in ethanol).

The absorbance of the blanks, standards and samples were measured against 0.3 M potassium sulfate at 505 nm using double beam spectrophotometer (GBC UV/VIS 918). The absorbance of the blanks and standards were measured before and after each sample batch.

The glucosinolate content in canola meal was calculated using the following formula:

$Cs = [As/K] \times [DF/Ws]$

$K = (\text{Mean Ast} - \text{Mean Ab}) / C$

Where

Ast = Absorbance of standards

Ab = Absorbance of blanks

C = Concentration of the sinigrin standards (µmol/ml)

Cs = Glucosinolate content of the sample (µmol/g)

As = Absorbance of the sample

DF = Dilution Factor

Ws = Weight of the sample (g).

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## STATISTICAL ANALYSIS

Standard Error of means were calculated for protein and glucosinolate content. Statistical comparisons between the treatments were made using SPSS version 10.00 one-way ANOVA. The normality was accepted at $p<0.05$ using Levine's and Bartlett's test (SPSS 2000). The significance was accepted at a probability of 5%.

## RESULTS AND DISCUSSION

*Moisture*

The results of moisture analysis are presented in Table 2. Moisture content of canola meal should be in the range of 6 to 10%. Above 10% moisture, glucosinolate hydrolysis will proceed rapidly, and below 6% moisture the myrosinase enzyme is only slowly activated by heat[11]. The moisture content in the study was 6.9 ± 0.1 %.

**Table2 Moisture Content of the Treated and Untreated Canola Meal Samples (%)**

| **Treatment** | | **Moisture content (%)** |
|---|---|---|
| **Soaking time (hours)** | **Temperature (°C)** | **Mean ± SE** |
| Control | Control | 6.9 ± 0.1 [a] |
| 4 | 40 | 66.4 ± 0.8 [b] |
| 4 | 60 | 63.4 ± 0.9 [b] |
| 4 | 80 | 52.2 ± 0.7 [b] |
| 8 | 40 | 68.5 ± 0.1 [b] |
| 8 | 60 | 68.0 ± 0.5 [b] |
| 8 | 80 | 66.6 ± 1.4 [b] |
| 12 | 40 | 71.1 ± 0.5 [b] |
| 12 | 60 | 67.8 ± 1.0 [b] |
| 12 | 80 | 64.5 ± 0.6 [b] |

Same superscript letters are not significantly different ($p < 0.05$)

As shown in Table 2, moisture content increased as the soaking time increased, and as the temperature increased, the moisture content decreased. The moisture contents in treated canola meal samples increased about 9 to 10 folds compared to the untreated canola meal samples.

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*Protein*

**Table 3 Protein Results of the Treated and Untreated Canola Meal Samples (Mean ± SE, %, n=3)**

| Treatment | | Protein content (%) |
|---|---|---|
| **Soaking time (hours)** | **Temperature (°C)** | **Mean ± SE** |
| Control | Control | 34.2 ± 0.2 [a] |
| 4 | 40 | 38.9 ± 0.3 [b] |
| 4 | 60 | 39.1 ± 0.0 [b] |
| 4 | 80 | 38.0 ± 0.6 [b] |
| 8 | 40 | 37.8 ± 0.5 [b] |
| 8 | 60 | 37.2 ± 0.8 [b] |
| 8 | 80 | 37.0 ± 1.5 [b] |
| 12 | 40 | 38.3 ± 0.9 [b] |
| 12 | 60 | 38.9 ± 1.0 [b] |
| 12 | 80 | 37.1 ± 0.2 [b] |

Same superscript letters are not significantly different ($p < 0.05$)

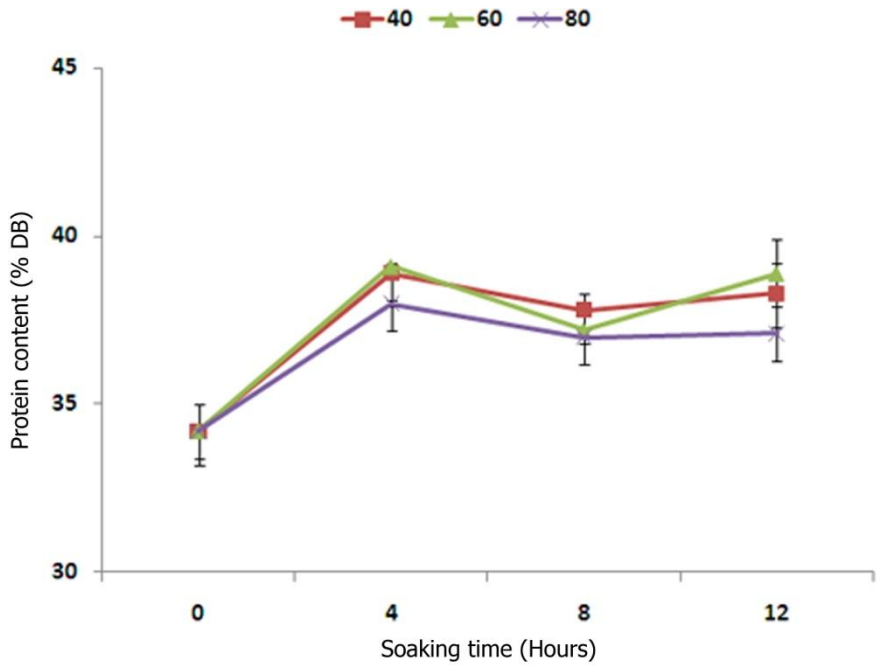


**Figure 1. The relationship between protein content (%) and soaking time (hours) followed by heating at different temperatures (Mean ± SE) in canola meal**

The protein analysis of raw canola meal showed that the protein content in the canola meal samples was 34.2% (db) and protein contents of the treated canola meal samples were in the range of 37.1 (%) (DM) to 39.1% (DM). However, there were no significance difference between treatments ($p<0.05$).

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All treated samples showed higher protein contents compared to the protein content of raw canola meal samples. The assumption of the higher protein content could be due to the soluble materials (which does not contain Nitrogen) leaching out into the water.

Data were assessed using Bartlett's test for homogeneity of variances[18] and graphical examination of the residuals. Bartlett's test is sensitive to departures from normality and it is frequently used for one-way analysis of variance. Post-hoc comparisons between treatment means were only assessed if significant F-test results from the analysis of variance were obtained.

*Glucosinolates content by rapid method*

The quantification of the total glucosinolate content was conducted by rapid method. The method provides accurate, quicker and simpler method than the HPLC method. Although HPLC permits measurement of the individual glucosinolates in a sample, it is time consuming, expensive, and requires sophisticated equipments and extensive calibration.

The result of glucosinolate analysis of the un-treated canola meal (control) showed that the canola meal used in the study contained 17.3 ± 1.0 µmol/g. The result of the experiment showed that soaking and heating significantly reduces the glucosinolate content in canola meal compared to the control (Table 4). The glucosinolate contents were reduced in all treatments. The differences between the treatments were not significant (One-way ANOVA, $p<0.05$). Therefore, lesser soaking time (4 hours) can be recommended.

**Table 4 Glucosinolate analysis (µmol/g) results (n=3)**

| **Treatment** | | **Glucosinolate content (µmol/g)** |
|---|---|---|
| **Soaking time (hours)** | **Temperature (°C)** | **Mean ± SE** |
| Control | Control | 17.3 ± 1.0 [a] |
| 4 | 40 | 10.7 ± 0.8 [b] |
| 4 | 60 | 9.7 ± 1.4 [b] |
| 4 | 80 | 9.6 ± 0.7 [b] |
| 8 | 40 | 10.4 ± 1.4 [b] |
| 8 | 60 | 8.6 ± 1.2 [b] |
| 8 | 80 | 11.2 ± 0.7 [b] |
| 12 | 40 | 10.3 ± 0.2 [b] |
| 12 | 60 | 11.0 ± 0.3 [b] |
| 12 | 80 | 9.8 ± 0.3 [b] |

Same superscript letters are not significantly different ($p<0.05$)

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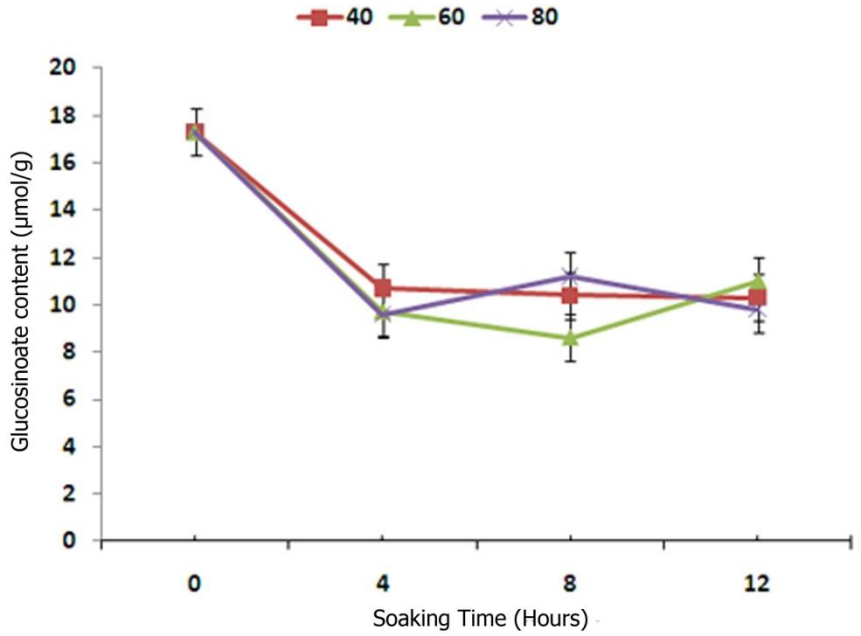


**Figure 2. The relationship between glucosinolate content (µmol/g) and soaking time (hours) followed by heating at different temperatures (Mean ± SE) in canola meal**

All treated canola meals samples had significantly lower glucosinolate contents compared to the non-treated canola meal. On the other hand, longer period soaking time had not significant difference on the glucosinolate content of samples. The result is in agreement with the result of the study conducted by Quinsac, *et al*. (1994)[19] and Fauduet *et al.* (1995).[20] They conducted cold aqueous extraction from the dehulled rapeseed meal. The results indicated that cold-water extraction could remove toxic factors in rapeseed meal while keeping good nutritional value.

Fauduet et al (1995)[20] used water to meal ratio of 6:1 (v:w) based on the convenience of stirring the rapeseed meal. The result of the experiment 94% of total glucosinolate was extracted at 17°C, and 99.5% was extracted at 95°C. As glucosinolates are affected by pH and temperatures, it is important to determine the range of temperatures where glucosinolate are easily extracted. Therefore, the present study was conducted at three different heating temperatures after soaking to determine the effect on glucosinolate content.

In the study conducted by Liu *et al*. (1994)[21] indicated that canola meal can be extracted with water to remove the toxic substances. Canola meal soaking in water results in leaching toxic substances including glucosinolates. They also found that severe toasting condition with high

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temperature and long duration would decompose glucosinolate. However, such treatement would reduce the quality of the protein.

The study conducted by Jensen et al. (1995)[22] involved toasting at 100°C for 15, 30, 60 and 120 min. The total glucosinolates decreased by 24, 46, 70 and 95% respectively. Although toasting for longer period of time (120 min) reduced more glucosinolate, the protein content also reduced significantly. Therefore in the present study, low temperature (40, 60 and 80 °C) was applied.

The hydrolysis of glucosinolates by endogenous myrosinase under moist conditions yields thiocyanates, isothiocyanate, nitriles and glucose. During the soaking process, glucosinolates are hydrolyzed and part of isothiocyanate, which is responsible for pungent flavor and bitter taste, is volatilized and thereby improves the feed intake.

## CONCLUSION

1. There was a significant reduction in glucosinolates in treated meal compared to untreated.
2. However, there was no significant difference in glucosinolates content between different soaking and heating treatments.
3. Therefore, 4 hours soaking followed by heating for 1 hour at 40°C was considered satisfactory to reduce glucosinolates from canola meal.

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## REFERENCES


1. Shahidi, F., Rapeseed and Canola: Global Production and Distribution, in F. Shahidi, (ed.) Canola and Rapeseed Production, Chemistry, Nutrition, and Processing Technology, New York : Van Nostrand Reinhold; 1990
2. Office of the Gene Technology Regulator, 'The Biology and Ecology of Canola (*Brassica napus*)'. Office of the Gene Technology Regulator, Australia. 2002
3. Department of Agriculture Western Australia, 'Weed Control in triazine-tolerant canola'. In Farmnote 52/96. Vol 2004, Department of Agriculture Western Australia, Perth. 1999
4. AgriFood Awareness Australia, 'GM Canola – Issues and Potential Market Impacts for Australia', vol. 2004, AgriFood Awareness Australia, Perth. 2004
5. Monsanto, 'Canola : Roundup Ready Canola', vol 2004, Monsanto, Perth.
6. Newkirk, R.W., Classen, H.,L. and Edney, M.J., Effects of prepress-solvent extraction on the nutritional value of canola meal for broiler chickens, *Animal Feed Science and Technology*, 2003; 104 : 111-119.
7. Naczk, M., Diosady, L.L. and Rubin, L.J., The phytate and complex phenol content of meals produced by alkanol-amoonia-hexane extraction of canola. *Lebensm-Wiss.u. Technol*; 1986, 19 : 13-16
8. Kracht, W., Jeroch, H., Daenicke, S., and Matzke, W., Effect of Dehulling Rapeseed on Feeding Value of Rapeseed Meal and – Cake for Poultry', in *10th International Rapeseed Congress*, The Regional Institute, Canberra, Australia. 1999a
9. Kracht W., Jeroch, H., Daenicke, S., Matzke, W., Hennig, U. and Schumann, W., 'Nutritional Evaluation of Rapeseed Cake from Dehulled Rapeseed Fed for Piglets and Growing-finishing Pigs', in *10th International Rapeseed Congress*, The Regional Institute, Canberra, Australia. 1999b
10. Xu, L. and Diosady, L.L., Removal of phenolic compounds in the production of high quality canola protein isolates. *Food Research International*. 2000; 33 : 725-731.
11. Canola Council of Canada, Canola Meal Processing. Available : [http://www.canola-council.org/pubs/meal4.html] (20 May 2004).
12. Glencross, B., Hawkins, W., and Curnow, J. Nutritional Assessment of Australian Canola Meals. I. Evaluation of canola oil extraction method and meal processing conditions on the digestible value of canola meals fed to the red seabream (*Pagrus auratus*, Paulin), *Aquaculture Research*, 2004, 35 : 15-24.
13. Mc Gregor, D., Blake, J. and Pickard, M., 'Detoxification of *Brassica juncea* with ammonia', in *6th international Rapeseed Congress*, Paris, 1983 pp. 1426 – 1430.
14. Larsen, L., Olsen, O., Ploeger, A. and Sorensen, H., Phenolic choline esters in rapeseed: possible factors affecting nutritive value and quality', in *6th International Rapeseed Congress*, Paris, 1983 pp 1577-1582.
15. Huang, S., Liang, M., Lardy, G., Huff, H.E., Kerley, M.S. and Hsieh, F. Extrusion Processing of rapeseed meal for reducing glucosinolates. *Animal Feed Science and Technology*, 1995, 56 : 1-9.
16. Association of Official Analytical Chemists, 'Official methods of analysis'. The Association of Official Analytical Chemists, Washington, D.C. 1996
17. Clerq, D.R. and Daun, J.K., Determination of the total glucosinolate content in canola by reaction with thymol and sulfuric acid. Journal of American of Chemists' Society, 1989; 66 : 788.
18. Snedecor, G. W. & Cochran, W. G. 1974, *Statistical Method*, The Iowa State University Press, Ames, Iowa. 1974
19. Quinsac, A., Lessire, M., Krouti, M., Ribaillier, D., Coic, J.P. Fauduet, H. and Rollin, P. Improvement in nutritive value of high or low glucosinolate rapeseed meal by aqueous extraction. Animal Feed Science Technology, 1994; 48 : 265-272.
20. Fauduet, H., Coic, J. P., Lessire, M., Quinsac, A., Ribaillier, D., Rollin, P., Rapeseed Meal Upgrading-Pilot Scale Preparation of Rapeseed Materials with High or Low Glucosinolate Content. Animal Feed Science Technology, 1995; 56 : 99-109.
21. Liu, Y.-G., Zhou, M.-Q. and Liu, M.-L. A survey of nutrients and toxic factors in commercial rapeseed meal in China and evaluation of detoxification by water extraction, *Animal Feed Science and Technology*, 1994, 45: 257-270.
22. Jensen, S.K., Liu, Y.-G. and Eggum, B.O., The effect of heat treatement on glucosinolates and nutritional value of rapeseed meal in rats, *Animal Feed Science and Technology*, 1995, 53 :17-28.