ORIGINAL PAPER

Anti-Nutritional Components, Fibre, Sinapine and Glucosinolate Content, in Australian Canola (Brassica napus L.) Meal

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Received: 28 February 2008 / Revised: 16 June 2008 / Accepted: 23 June 2008 / Published online: 21 August 2008 AOCS 2008

Abstract Canola meal is highly regarded as a component of animal feed with a high protein content and a desirable amino acid profile. The presence of some components, in particular glucosinolates, sinapine and fibre, affects the value of the meal and reduces the amount that can be used in animal feed formulations. Glucosinolates in traditional cultivars (rapeseed) had very high amounts and this severely limited the usefulness of the meal. Canola breeding programs have successfully reduced glucosinolate content to trace amounts. However sinapine remains at levels sufficiently high to cause problems, particularly in poultry feed. The relatively high fibre level in canola also reduces the value of the product for animal feed. This study has determined the level of sinapine, glucosinolates and fibre in current cultivars of canola in Australia to illustrate advances made by breeding programs and limitations which still remain to raise the usefulness of a potentially valuable feedstock. Although glucosinolate levels in meal were shown to have been reduced to $11 \mu \text{mol/g}$ in some cases, sinapine remained at traditional levels of about 12– 15 g/kg and neutral detergent fibre levels were about 30– 40%. These issues are important priorities for canola breeders.

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Keywords Canola Meal Fibre NDF ADF . Crude fibre · Sinapine · Glucosinolate · Anti-nutritional

Introduction

Canola is grown principally as a source of edible oil but the meal component, remaining after the oil has been extracted, is a good source of protein for livestock feed. In order to be classified as canola, the oil in the seed must contain $\langle 2\%$ erucic acid and the meal must contain $\langle 30 \rangle$ umol of four individual glucosinolates per gram of meal [[1\]](#page-7-0). However, the presence of other anti-nutritional components in the meal, such as sinapine, can detract from the usefulness of canola meal as a dietary supplement [\[2](#page-7-0)]. Canola meal has relatively poor digestibility when compared to other food sources, such as soybean meal, due to its higher fibre content.

Sinapine is a choline ester of sinapic acid [[3\]](#page-7-0) which is important in plants for the biosynthesis of lignin and flavonoids [\[4](#page-7-0)]. Sinapine has several undesirable properties as a constituent in animal feeds. It is a bitter tasting compound, making it less palatable to animals, while its presence in the diet of certain brown egg laying hens at levels exceeding 1 g/kg leads to a fishy odour or taste in the eggs $[5]$ $[5]$.

Prior to the development of canola from rapeseed, the presence of glucosinolates was the major limiting factor in the use of rapeseed for livestock food [[6\]](#page-7-0). Glucosinolates are sulphur containing glycosides which have been found to reduce animal performance, impair thyroid function in growing animals and foetuses as well as other toxic effects [\[7](#page-7-0), [8\]](#page-7-0). As a result of selective breeding programs, the concentration of glucosinolates has been reduced to trace levels.

Fibre including cellulose, pentosans and lignin from cell walls, is mainly present in the hulls of canola. High fibre contents in animal feeds decrease its digestibility, thus decreasing the value of the feed.

The purpose of this study was to investigate the levels of the anti-nutritional components, glucosinolates, sinapine and fibre, in Australian canola and evaluate the degree of improvement through Australia's canola breeding program. The Australian Oil Research Laboratory at Wagga Wagga, NSW, Australia has been central to the analysis of samples from national canola breeding programs for over 15 years.

Materials and Methods

Canola samples were obtained in 2002 and 2003 from the Australian National Brassica Improvement Project trials funded by the Grains Research and Development Corporation. These samples were from sites in Western Australia (Wongan Hills, Newdegate, Katanning), South Australia (Minnipa, Bordertown, Struan) and New South Wales (Wagga Wagga, Moree, Tamworth). Canola trial samples were taken from early maturing and mid maturing cultivars. Samples (96) of other Brassica species were obtained from the Australian Temperate Field Crops Collection, Horsham, Australia.

Analytical Methods

All solvents were analytical grade and were purchased from Lomb Scientific (Taren Point, Australia).

Sinapine Extraction

Canola seed (10 g) was ground for 10 s (twice) using a coffee grinder. The oil was removed from the sample using a Soxhlet apparatus (Petroleum ether, $40-60$ °C, 16 h). The meal was then dried and reground using a coffee grinder prior to further analysis.

The meal (0.04 g) was accurately weighed into 2-mL plastic micro centrifuge tubes. Ethanol (70%, 1 mL) was added and the samples were mixed for 2 s to ensure thorough wetting of the sample. The tubes were shaken for 1 h on an end-over-end shaker at 5 rpm. The samples were centrifuged at 13,000 rpm for 5 min using a high-speed centrifuge. The clear supernatant was drawn off with a 1-mL syringe, filtered using a 0.45 µm nylon syringe filter then transferred to HPLC vials for analysis. Extracts were stored at 4° C until analysed [[9](#page-7-0)].

Sinapine Standard Preparation

Sinapine bisulphate standard was extracted according to the method of Clandinin [\[10\]](#page-7-0). The oil was removed from 500 g mustard seed (Sinapis alba L.). The defatted meal was then extracted with 95% ethanol on a soxhlet apparatus for 4 h. The alcoholic extract was concentrated to a thick syrup then diluted to 500 mL with distilled water. Potassium thiocyanate (20%) (100 mL) was added and the solution stored at 4 °C for 48 h. The sinapine thiocyanate crystals were recovered by centrifugation and decanting. The wet crystals were dissolved in 200 mL of hot (75 \degree C) 95% ethanol and stored at 4 \degree C for 24 h. The crystals were collected by filtration.

The sinapine thiocyanate was then converted to sinapine bisulphate. Sinapine thiocyanate was dissolved in 400 mL of hot ethanol. Concentrated sulphuric acid (5 mL) was added slowly and the solution refrigerated for 24 h. The crystals were collected by filtration then dissolved in 300 mL water. Concentrated sulphuric acid was slowly added for a second time then refrigerated for 24 h and the crystals again collected on filter paper. The crystals were dissolved in 400 mL of hot ethanol, cooled and refrigerated for 24 h. The crystals were collected on filter paper, dried at room temperature in a desiccator and stored at 4° C. This standard was used to produce a calibration curve range of 250–1,000 mg/L in 70% methanol.

Sample Analysis

The samples were analysed using a Waters HPLC system including two 515 pumps with mixer, a 717 Autosampler, column oven with temperature control and a 484 UV-Vis detector. The system was controlled by Millennium 32 software.

A Vydac protein and peptide C18 column, 0.46 cm \times 25 cm, was used. Solvent A was 0.1% trifluoroacetic acid (TFA) in water and solvent B was 0.1% TFA in acetonitrile. A constant flow rate of 1 mL/min was used throughout the analysis with a column oven temperature of 40 °C. The gradient used was as follows: 100% solvent A for 5 min, 62.5% solvent A/37.5% solvent B in 50 min, 100% solvent A in 1 min. A comparison of various wavelengths between 210 and 330 nm showed the greatest response for the sinapine peak to be at 330 nm, consistent with the findings of Cai and Arntfield [[11\]](#page-7-0). The sinapine peak is detected at \sim 30 min.

Crude Fibre

Crude fibre was determined by digesting the sample in sulphuric acid followed by sodium hydroxide in an Ankom 220 Fibre Analyzer according to the official method of the American Oil Chemists Society, AOCS Ba 6a-05 [\[12](#page-7-0)].

Neutral Detergent Fibre

Neutral detergent fibre (NDF) was determined by digesting the sample in a detergent solution using an Ankom 220 Fibre Analyzer according to the official method of the Australian Fodder Industries Association Method 1.9A(a) [\[13](#page-7-0)].

Acid Detergent Fibre

Acid detergent fibre (ADF) was determined by digesting the sample remaining from the NDF analysis with sulphuric acid and cetyl trimethylammonium bromide, using an Ankom 220 Fibre Analyzer according to the official method of the Australian Fodder Industries Association Method 1.8A(a) [[14\]](#page-7-0).

Glucosinolates

Total glucosinolates were measured as glucose from the hydrolysis of the glucosinolates according to the official method of the Australian Oilseeds Federation Method 4- 1.22 $[15]$ $[15]$ and presented as μ mol/g oil-free meal at 10% moisture.

Statistical Analysis

The experiment was performed using a randomized block design. Data were analysed using GenStat version 9.1 (Lawes Agricultural Trust, UK). Analysis of variance was used to determine the differences among means.

Results and Discussion

This study utilized eight canola cultivars, consisting of four early maturity types and four mid maturity types, to

determine current levels of antioxidants and anti-nutritional components for monitoring whether the levels are adequately low after many years of plant breeding and improvement. Samples of the eight cultivars were grown over 2 years and at nine different sites to determine if seasonal differences or site differences were responsible for quality differences.

Glucosinolate Content

Total glucosinolate content ranged in current cultivars from 11 to 34 µmol/g of oil and moisture free meal, with most being within the Canola Council limit for canola of 30 µmol/g of four specific glucosinolates/g of meal. Despite this, glucosinolates continue to cause problems for industry with the odour from hydrolyzed isothiocyanates being an issue for processors based in major cities. Perceptions of feed manufacturers on the effect of glucosinolates is a factor limiting canola meal utilization in animal feed to low proportions. The aim of breeders is to develop cultivars with a zero glucosinolate content to remove limitations caused by their presence.

Site Effect

Glucosinolates were not significantly affected by site (Table 1). Other studies have reported similar results [\[8](#page-7-0)]. The mean range for the mid maturity types (13–18) was lower than the range for the early maturity samples (19– 27), Similar studies have shown similar ranges [[8\]](#page-7-0). A large percentage of relative standard deviation (71%) was evident for the Moree site, due to cultivar AGC202 at that site

Table 1 Effect of site on mean value of quality components of early and mid maturity canola lines

All results in oil/moisture free meal (percentage of relative standard deviation)

NDF neutral detergent fibre, ADF acid detergent fibre

$N = 20$	Crude fibre $(\%)$	NDF $(\%)$	ADF $(\%)$	Sinapine (g/kg)	Glucosinolates $(\mu \text{mol/g})$
2002 Early	10.7(10)	31.4 (14)	18.1(15)	14.8 (11)	25(53)
2003 Early	11.8(15)	33.8(14)	18.5(14)	13.9(10)	18 (24)
p -Value	0.029	0.105	0.655	0.064	0.027
2002 Mid	11.8(10)	34.3(16)	17.5(13)	13.6(11)	15(22)
2003 Mid	13.2(9)	37.1(9)	20.2(12)	14.5(11)	15(28)
p -Value	< 0.001	0.065	< 0.001	0.050	0.824

Table 2 Effect of year on quality components on early and mid maturity lines of canola

All results in oil/moisture free meal (percentage of relative standard deviation)

NDF neutral detergent fibre, ADF acid detergent fibre

showing a relatively high glucosinolates level in 2002. It is not clear why this occurred.

Year Effect

Glucosinolates concentration was significantly influenced by year, or growing season, for the early maturity varieties with a mean of $25 \mu mol/g$ in 2002, compared with 18 μmol/g in 2003 (Table 2). No significant differences were found for the mid maturity samples ($p = 0.824$).

Cultivar Effect

Glucosinolates were significantly affected by cultivar $(p<0.001$ for early and mid maturity types). The mean glucosinolates concentration in the early maturity variety AGC 202 was 34 µmol/g while the other cultivars were $17-18$ μ mol/g. The mean range for the glucosinolates in the mid maturity cultivars was $11-17 \mu$ mol/g, with Rainbow and Lantern showing the highest value (Table 3). These results are very similar to those reported in other studies [[8\]](#page-7-0), with Brand et al. showing a range of 17– 19 µmol/g of glucosinolates in a number of canola cultivars grown in South Africa.

Sinapine Content

Sinapine has been basically ignored in breeding programs due to the lack of genetic variation within B. napus lines where the levels are generally around 13–15 g/kg. In this study we found sinapine to be within the range of 10–18 g/ kg, a limited range despite the different cultivar types utilized and the growing conditions for each trial.

Site Effect

Sinapine concentration was not affected by growing site, similar to other studies [\[8](#page-7-0)], for either the early or mid maturing canola (Table [1\)](#page-2-0). The mean range of sinapine concentration in the early maturity samples was 13.2–15.2 g/kg and for the mid maturity samples 12.9– 14.7 g/kg.

Year Effect

Sinapine concentrations were found to be unaffected by the growing season in both the early maturity and mid maturity samples ($p = 0.064$ and 0.050 respectively) (Table [2](#page-3-0)).

Cultivar Effect

The sinapine concentration in the early maturity samples was significantly affected by cultivar ($p < 0.005$), as found in other studies [\[8](#page-7-0)]. Mean results ranged from 13.0 to 15.1 g/kg (Table [3\)](#page-3-0). Sinapine in mid maturity samples were also affected by cultivar ($p < 0.001$), with a mean range of 12.4–15.2 g/kg. These ranges are slightly higher than those reported by other researchers [[8\]](#page-7-0).

Crude, Acid Detergent and Neutral Detergent Fibre

A reduction in fibre levels has been seen as a method of increasing the levels of oil and protein. Despite this, selection for fibre has not been a priority for Australian breeding programs. The ongoing selection of cultivars for higher oil and protein has indirectly resulted in some decrease in fibre levels. Further selection for reduced fibre would appear to be desirable to improve canola meal quality.

Site Effect

The mean values for crude fibre content in the samples studied were between 9.9 and 13.0% in oil and moisture free meal. Crude fibre content in the early maturity cultivars were shown to be significantly different ($p < 0.001$), however this was not the case with the mid maturity cultivars ($p = 0.275$). In contrast to crude fibre, NDF was found to be affected by site for the mid maturity cultivars $(p<0.001)$, and there was no significant effect of growing site on the early maturity cultivars. There were no significant effects of site on the ADF content of the samples.

Year Effect

Crude fibre, NDF and ADF were not significantly affected by the season for early maturity samples. However, in mid maturity types, crude fibre and ADF were significantly affected (both $p < 0.001$) (Table [2\)](#page-3-0).

Cultivar Effect

There was a significant cultivar affect only for ADF for the early maturity cultivars ($p < 0.002$) with crude fibre and NDF not significantly affected (Table [3\)](#page-3-0). Outback had the maximum mean for all of these components. Crude fibre,

ADF and NDF were not significantly affected by cultivar for the mid maturity cultivars.

Alternative Species

The lack of variation for sinapine, fibre and glucosinolate content in current Australian cultivars of B. napus indicates that the necessary variation may need to be provided by other species. To determine if this variation exists, a selection of 96 samples from the Australian Temperate Field Crops Collection, Horsham, Australia were analysed for sinapine and glucosinolates content (Table [4\)](#page-5-0).

Glucosinolates

There were no clear trends in the material analysed from the Australian Temperate Field Crops Collection. Although B. napus cultivars from Australia and Sweden were the lowest in glucosinolates, cultivars of B. napus were spread across the range from 9 to 169μ mol/g. The cultivated species showed large ranges between the highest and lowest levels with B. carinata (64–167 μ mol/g); B. juncea (85–202 μ mol/g); *B. nigra* (132–198 μ mol/g) and *B. rapa* ($29-195 \mu$ mol/g). The overall range for glucosinolates was from 9 μ mol/g, being for a *B. napus* sample from Sweden, to 202 μ mol/g in a *B. juncea* sample from India.

Sinapine

The range for sinapine was significant both between and within the different species. Of interest was B. tournefortii which had the lowest level of sinapine at 6.0–6.8 g/kg and was three of the lowest five samples for sinapine content. For cultivated species, the ranges were B. carinata (8.5–14.5 g/kg); B. juncea (7.3–14.3 g/kg); B. napus $(7.4–16.2 \text{ g/kg})$; *B. nigra* $(6.7–12.2 \text{ g/kg})$ and *B. rapa* $(5.2–12.2 \text{ g/kg})$ 14.7 g/kg). The overall range for sinapine concentration was 5.2 g/kg in a *B. rapa* sample to 16.2 g/kg in a *B. napus* sample from Germany. This is similar to the findings of Zum Felde et al. $[16]$ $[16]$ and Matthaus $[17]$ who found a similar range (3.2–12.7 g/kg) of sinapine in *B. napus.*

Breeding Progress

From the comparison of the range for glucosinolates content in alternative species and the levels shown in Tables [1](#page-2-0), [2](#page-3-0) and [3](#page-3-0) in this study, it is clear that breeding has had a substantial affect on reducing glucosinolate content to very low levels. The maximum level found in commercial cultivars of B . *napus* in this study was $34 \mu m o l/g$ of total glucosinolates in oil and moisture free meal and a mean value of 18 µmol/g across eight cultivars grown at

Table 4 Glucosinolate and sinapine content of 96 Brassica samples

Name	Taxon	Origin	Glucosinolates (µmol/g)	Sinapine (g/kg)
B.CR.-47	Brassica carinata	Unknown	117	11.8
Ethiopia B	Brassica carinata	Unknown	118	11.9
Mestnaja	Brassica carinata	Unknown	128	11.4
PI 183437	Brassica carinata	Unknown	138	12.2
PI 184290	Brassica carinata	Unknown	64	13.3
PI 193761	Brassica carinata	Unknown	110	13.7
$UCD-16$	Brassica carinata	Unknown	130	13.7
$UCD-18$	Brassica carinata	Unknown	131	14.5
BRA 1156/85	Brassica carinata	Unknown	167	13.4
BRA 927/72	Brassica carinata	Ethiopia	128	12.0
BRA 489/77	Brassica carinata	Ethiopia	125	13.0
CPI99838	Brassica carinata	Unknown	143	8.5
CPI99847	Brassica carinata	Unknown	138	11.1
CPI100551	Brassica carinata	Unknown	137	11.4
CPI100563	Brassica carinata	Unknown	155	9.7
BCA1	Brassica carinata	Australia	95	12.4
BFR 2	Brassica fruticulosa	Australia	104	9.5
Brassica incana Ten.	Brassica incana	Italy	183	10.4
Commercial	Brassica juncea	Canada	131	11.1
Ekla	Brassica juncea	Australia	140	9.4
CSIRO 81792	Brassica juncea	Australia	166	10.4
MRS88 19	Brassica juncea	India	202	10.0
MRS88 20	Brassica juncea	Turkey	152	10.5
MRS88 59	Brassica juncea	UK	156	14.3
MRS88 64	Brassica juncea	Cuba	149	9.8
MRS88 65	Brassica juncea	Puerto Rico	122	13.2
MRS88 70	Brassica juncea	Afghanistan	164	9.6
MRS88 72	Brassica juncea	Pakistan	166	10.7
K 1072	Brassica juncea	Afghanistan	164	10.9
MRS88 254	Brassica juncea	Bangladesh	181	8.9
Aurea	Brassica juncea	Germany	157	10.9
Orient Yellow	Brassica juncea	USA	148	10.6
PI 478325	Brassica juncea	China	143	11.3
Neosypa.	Brassica juncea	Czech Republic	139	10.4
MRS88 361	Brassica juncea	Afghanistan	85	7.3
Orient, Must, D	Brassica juncea	Canada	182	10.2
Siromo	Brassica juncea	Australia	146	11.1
B. integrifolius	Brassica juncea var. integrifolia	India	110	10.2
Juno	Brassica napus	Sweden	111	14.1
Doral	Brassica napus	Germany	144	15.6
Belinda	Brassica napus	Germany	168	16.2
Tamara	Brassica napus	Germany	142	14.4
Jaspard	Brassica napus	France	169	11.7
Mikado	Brassica napus	UK	133	15.5
Taparoo	Brassica napus	Australia	14	10.8
Reston	Brassica napus	Canada	35	13.0
Pivot	Brassica napus	Canada	21	12.0
Golden	Brassica napus	Canada	117	10.6
Topas	Brassica napus	Sweden	9	13.1

Table 4 continued

Samples were obtained from the Australian Temperate Field Crops Collection, Horsham, Australia. All results in oil/moisture free meal. Single analysis only

nine sites over 2 years. This is substantially different to that found in the traditional levels in B. napus as illustrated in Table [4](#page-5-0). The variation between sites, seasons and cultivars indicates that more can be done to reduce the glucosinolate content. This and additional data from years of canola analysis [18] illustrates that glucosinolates are well within Canadian Canola Council specifications for canola.

Sinapine reductions have not been as successful as for glucosinolates concentration due to the lack of variation within B. napus cultivars and also due to the lack of breeding effort. It is clear from Table [4](#page-5-0) that there is considerable variation both within and between species and therefore selection for lower levels is possible.

Fibre content has not been a priority in breeding and any reduction in fibre has been an indirect effect of selections for increased oil and protein. The lack of variation between the cultivars studied is disappointing and suggests that selection for low fibre levels may be difficult.

Continued efforts by plant breeding programs will be focussed on increasing yield and oil content, the main economic factors for growers. However, increased demand for canola meal with improved sinapine, glucosinolates and fibre will also have an economic benefit for growers.

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