International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 8(2): 253-264 June 2015
DOI Number: 10.5958/2230-732X.2015.00032.7
©2015 New Delhi Publishers. All rights reserved

GENETICS

Biochemical Investigation on Antioxidative and Antinutritional Characters of Yellow Seeded *Brassica* Genotypes for Quality Assessment

H. Punetha[1*], Dinesh Pandey[2], Pooran Bhatt[1], Heena Sagar[1] and B.Rawat[1]

[1]Department of Biochemistry, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar -263 145, U. S. Nagar, Uttarakhand, India.
[2]Department of Molecular Biology and Biotechnology, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar -263 145, U.S. Nagar, Uttarakhand, India.

*Corresponding author: punetha_hp@rediffmail.com

Paper No. 316 Received: 21 April 2014 Accepted: 21 May 2015 Published: 28 June 2015

Abstract

Yellow seeded *Brassica* is one of the most important oilseed crop cultivated in many parts of the world. The oil is consumed predominantly as edible oil and the defatted meal cake is utilized as animal/poultry feed. In the present study, intact seeds and defatted meal of 12 yellow-seeded *Brassica* genotypes evaluated for the presence of anti-oxidative and anti-nutritive factors. The maximum phenolic, ortho-dihydroxy phenols, flavonoid contents observed for Bio 39(16.946 mg/g), Bio 30(2.38 mg/g) and Bio 39(8.282 mg/g) repectively. The crude fibre, phytic acid and glucosinolate contents were found to be minimum in Bio 21 (6.95%), Bio 2 (3.632%) and Bio 38 (54.324 μmole/g) respectively. Vitamin C content was maximum in Bio 36 and Bio 3. The α-tocopherol content was observed to be maximum Bio 2 (61.875 mg/100g). The total antioxidant activity varied from 5.95 mg AAE/g in Bio 28 to 14.395 mg AAE/g in Bio 39. The DPPH radical scavenging activity was observed to be maximum in Bio 30 and reducing power was found to be maximum in Bio 21(0.867 ± 0.023). The present findings may be utilized for determining the quality status to ascertain their potential for development of better cultivar.

Highlights

- Evaluation for antioxidative and antinutritional characters of yellow- seeded Brassica genotypes.
- The minimum contents of antinutritional factors such as crude fiber, phytic acid and glucosinolates were observed in Bio21, Bio 2 and Bio 38 respectively.
- Maximum antioxidant level observed in Bio2, Bio 28, Bio 30 and Bio 21.
- Present finding will be useful for understanding quality status for the development of better cultivars.

Keywords: Yellow seeded Brassica, meal, antioxidative, antinutritional, quality

Rapeseed-mustard belongs to the family Brassicaceae or Cruciferae, consisting 350 genera and 3,500 species (Sasaki and Takahashi 2002). It is an important oilseed crop and currently ranked as the world's third important oil crop in terms of production and area. The annual production of Rapeseed-mustard is 63.04 million tons of seed from an area of 34.33 million hectares (FAO 2013). India is among the largest producer and consumer of vegetable oils in the World. Indian vegetable oil economy is the fourth largest in the world next to USA, China and Brazil (Singh *et al.* 2012). Oilseeds are next to food grains

in terms of area, production and value accounted for almost 5% of Gross National Product (GNP) in 2006. The oil content in rapeseed is around 40%, and the defatted meal is rich in protein and usually used as animal feed (Yue *et al.* 2014). Rapeseed meal has high protein content (~ 40%) and nutritional value, it is still underutilized and mainly used as fertilizer (Hashmi *et al.* 2010). Plants have played a significant role in providing the human race with remedies (Kaur *et al.* 2014), and rapeseed-mustard is not an exception. The defatted meal is used as rich source of proteins and minerals to the animals and poultry with a well-balanced amino acids and vitamin E. The major anti-oxidants present are vitamins E and C, carotenoids, and phenolic compounds, especially flavonoids. Flavonoids, as well as vitamin C showed a protective activity to α-tocopherol in human LDL, and they can also regenerate vitamin E, from the α-chromanoxy radical (Davey *et al.* 2000). These *anti-oxidants* scavenge radicals and inhibit the chain initiation or break the chain propagation (the second defense line). However, its utilization in animal food is restricted due to the presence of different anti-nutritional factors such as glucosinolates, phytic acid and crude fibres. Therefore, it is imperative to screen the different yellow-seeded *Brassica* genotypes for their proper utilization as a rich source of nutrient for the animal as well as human consumption.

Materials and Methods

Preparation of defatted meal

For the preparation of a defatted meal, the thimble of muslin cloth was used for packing of crushed seeds. This thimble placed in Soxhlet and in the presence of petroleum ether, as a solvent, defatting was done at 50°C. The extraction of oil with solvent and the defatted meal, which is retained in the thimble, was used for different biochemical analysis.

Preparation of methanolic extract

The defatted meal (0.1 g) mixed with 2.0 ml of 80% methanolic solution was added to it, after that this mixture was homogenized properly, and then it was centrifuged at 3000 rpm. The supernatant was collected, and the final volume was made up to 2.0 ml.

Total Phenol Content

The method developed by Singleton and Rossi 1965 followed for the total phenol content. The absorbance read at 765 nm. The standard curve was established using various concentrations of gallic acid, and results were expressed as gallic acid equivalent.

Ortho-dihydroxy phenol content

Ortho-Dihydroxy phenols in the extract were determined by Arnow's method. A Standard curve prepared from catechol at different concentrations, and the amounts were calculated with the help of calibration curve and expressed in mg/g of the sample.

Flavonoid Content

The total flavonoid content estimated by the method of Choi *et al.* (2006). The absorbance of color developed at 510 nm.

Total Antioxidative activity

Total *anti-oxidant* content was estimated by the method of Prieto *et al.* (1999). The assay based on formation of a green phosphate/Mo (V) complex under acid condition. The absorbance of the sample was measured at 695 nm against a blank. The anti-oxidant activity was expressed about that of ascorbic acid.

Vitamin C content

Vitamin C was estimated by the method of Law *et al.* (1983). The colour intensity read at the absorbance of 465 nm. The results expressed as mg ascorbic acid/100 g.

Tocopherol content

The tocopherols were estimated according to Backer *et al.* (1980) with some modifications. The absorbance of the mixture read at 522 nm, 50 sec after adding the ferric chloride solution. The tocopherol used for making the standard curve.

DPPH Free Radical-Scavenging Activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity of methanolic extracts was determined, following the procedure described by Yen and Duh, (1993).The absorbance was taken at 517 nm using UV-spectrophotometer. The percentage DPPH Radical scavenging activity was calculated by the following equation.

Reducing Power Activity

The reducing power of methanolic extract of the defatted meal was measured according to Yen and Duh, (1993) with some modifications. The absorbance was recorded immediately at 700 nm using the spectrophotometer.

Crude Fibre content

Crude fiber content was estimated by the method of Ahuja *et al.* (1999). The absorbance of color developed in the reaction mixture read at 590 nm.

Phytic Acid content

The method of Davies and Reid (1979), was followed for estimation of Phytic acid. The colour intensity was read at 465 nm against amyl alcohol blank after 15 minutes. The test tubes with different concentrations of sodium phytate run along with the sample. The results expressed as g /100 g of the defatted meal.

Total Glucosinolate Content

Total glucosinolates estimated by using the colorimetric method of Kumar *et al.* (2004). The estimation of glucosinolates based on the formation of a complex between hydrolytic products of glucosinolates and sodium tetrachloro palladate (II), colorimetrically. The intensity of colour measured at 405 nm using micro scan ELISA Reader.

Amount of Total Glucosinolates was calculated by using following regression equations:

A. Total glucosinolate conc. (μmol/g) = $(OD_{405} - 0.046) \times 82$

B. Total glucosinolate conc. (μmol/g) = $(OD_{405} - 0.169) \times 115.74$

If the absorbance of the sample was greater than 0.8 then equation B will be used for estimating total glucosinolate content, otherwise equation A will be utilized.

Results and Discussion

It was observed that Bio 39 genotype had higher total phenolic content (16.946 mg/g) and Bio 22 (11.313 mg/g) have the lowest (Figure 1). The phenol content varied significantly among the different yellow seeded *Brassica* genotypes. Other genotypes having intermediate level of phenol contents were such as Bio 30 (14.18 ± 0.17 mg/g), Bio 38 (13.36 ± 0.12 mg/g), Bio 28 (13.27 ± 0.05 mg/g), Bio 37 (13.23 ± 0.02 mg/g), Bio 36 (13.00 ± 0.07 mg/g). Our results were found to be similar to the results of Shahidi and Naczk, (1992), who reported the total phenolic compounds content in rapeseed meal at the level of 10.802 – 18.07 mg/g. Phenolics are the most abundant secondary metabolites of plant origin which form an important part of both human and animal diets (Iqbal Muhammad *et al.* 2015). They are important factors when considering rapeseed meal as a source of human food-grade protein because they contribute to the dark colour, bitter taste, and astringency of rapeseed protein products (Shahidi and Naczk 1992). Also, phenolics and their oxidized products can form complexes with essential amino acids and proteins, responsible for decreasing its nutritional potential.

The Ortho dihydroxy phenols content was found to be maximum in Bio 30 (2.38 ± 0.01 mg/g) followed by Bio 39 (2.06 ± 0.01 mg/g) and Bio 2 (1.59 ± 0.02 mg/g) whereas the minimum content was present in Bio 21 (1.16 ± 0.02mg/g) (Figure 2).

The flavonoid content is expressed as mg/g and varied from 3.898 mg/g in Bio 21 to 8.282 mg/g in Bio39. The flavonoid content varied significantly among the *Brassica* genotypes (Fig: 3) under study. In other genotypes, content was observed in Bio 36 (7.31 ± 0.00 mg/g), Bio 3 (6.94 ± 0.12 mg/g), Bio 30(6.82 ± 0.97mg/g). In *Brassica napus* var *napus*

inflorescence, the flavonoid content was found to be 221.01 ± 6.60mg/100 g dry weight (Batista *et al.* 2011). Flavonoids have interesting biological activities due to which they are being used in numerous medical treatments (Morton *et al.* 2000), cancer-prevention (Birt *et al.* 2001), cardiovascular system protection and inhibition of oxidative damage (Williams *et al.* 2004).

The total anti-oxidants content is expressed as mg AAE/g varied from 5.95 mg/g in Bio 28 to 14.395 mg/g in Bio 39. The total anti-oxidants content varied significantly among the *Brassica* genotypes (Fig: 4). In Bio 36, Bio 2 and Bio 18, the total anti-oxidants content was observed to be 10.56 ± 1.06 mg/g, 10.33 ± 0.50 mg/g and 9.92 ± 0.12 mg/g, respectively. The total antioxidant capacity results from the contribution expressed by the whole mixture, namely phenolic compounds with the flavonoid fraction, anthocyanins, glucosinolates and vitamin C (De Nicola *et al.* 2013).

Ascorbic acid content varied from 40.665 mg/100g in Bio 2 to 144.665 mg/100g in Bio 36 and Bio 3(Fig5). The intermediate vitamin C content was observed in Bio 30 (142.66 ± 1.88mg/100g), Bio 38 (138.66 ± 1.88mg/100g), Bio 37(129.33 ± 16.97mg/100g). Vitamin C content was found to be 68.3 mg/100 g for canola (Funda and Murat, 2011). The Vitamin C content influenced various factors, sowing time as well as harvesting date (Kim and Ishii, 2007). Block *et al.* (2004) found that vitamin C can reduce levels of C-reactive protein (CRP), a marker of inflammation and possibly a predictor of heart disease.

Tocopherols are important *anti-oxidants* and variation for its content in 12 yellow-seeded *Brassica* genotypes presented in Figure 6. In the present study, the maximum tocopherol content in the defatted meal of yellow seeded *Brassica* genotypes observed in Bio 2 (61.875 ± 2.411 mg/100g) followed by Bio 38 (44.464 ± 3.482 mg/100g). Gupta *et al*. (2012) reported that the tocopherols in *Brassica juncea* ranged from 3.229 to 125 mg/100g. Tocopherols provide the protection against free radicals which may be responsible for ageing process, cancer, cardiovascular diseases, cataract and retard lipid oxidative rancidity in foods and increasing the shelf life of oils having poly-unsaturated fatty acids (Lai *et al.* 2001).

DPPH scavenging activity of methanolic extracts from all 12 yellow-seeded *Brassica* genotypes meal presented in Figure 7.The highest free *radical-scavenging* activity found in Bio 30 (50.251 ± 1.112%) followed by Bio 37 (44.724 ± 0.558%), Bio 18 (43.216 ± 1.827%) and Bio 39 (43.150 ± 0.950%) at the concentration of 200 µg/ml. As the concentration of the sample was increased, the percentage radical scavenging was also increased. Marina *et al.* (2009) reported that the IC_{50} value for rapeseed meal was 0.009 mg/ml for 70% methanolic extract. It is well accepted that the antioxidant properties of edible portions of *Brassica* species are mainly due to their polyphenolic components (Ayaz *et al*. 2008).There is an increasing interest in natural food additives that can function as natural antioxidants(Kaur *et al.* 2014).

All investigated yellow-seeded *Brassica* have reductive capabilities (reducing power) and potential antioxidant activity. Maximum reducing power activity was found in Bio21 (0.867 ± 0.023) followed by Bio36 (0.634 ± 0.008) and Bio19 (0.628 ± 0.015) at the concentration of 2.0 mg/ml, whereas the minimum reducing power activity was found in Bio18 (0.251 ± 0.010) followed by Bio38 (0.382 ± 0.011) and Bio 2 (0.435 ± 0.003) at the concentration of 2.0 mg/ml (Figure 8). Marina *et al.* (2009) reported that the absorbance of 70% methanolic extract of rapeseed meal was 0.484 at the concentration of 0.5 mg/ml.

The Crude fibre content varied from 6.95% (in Bio 21) to 13.49% (in Bio 37). The Crude fibre content varied significantly among the *Brassica* genotypes (Figure 9) under the present study. Content of crude fiber was also observed in Bio 2 (11.67 ± 0.06%), Bio 18 (9.98 ± 0.01%), and Bio 19 (9.56 ± 0.00%). Thanaseelaan (2013), reported the level of crude fiber in rapeseed meal was 9.14 ± 0.25% on dry weight basis. According to AOAC report the crude fiber content was reported to be in the range from 7.4 - 13.8% on dry seed

basis. The high fiber content reduces the amount of metabolizable energy of the rapeseed meal and thus decreasing the feed value. The digestibility and availability of rapeseed meal nutrients are limited by both its composition (high dietary and crude fibre level) and processing (toasting) (Pastuszewska *et al.* 2003).

In the present study, phytic acid content was found to be varied from 3.632% in Bio 2 to 5.404% in Bio 36 (Figure 10). Phytic acid content was also observed in Bio 38 (5.39 ± 0.00%), Bio 37 (5.39 ± 0.01%), and Bio 18 (5.33 ± 0.005%). Phytate is an antinutrient because it chelated divalent minerals and reduced their physiological availability (Hurrell 2003).

The glucosinolate content expressed as µmol/g varied from 54.324 µmol/g (Bio38) to 125.28 µmol/g (Bio36) (Figure 11). Kumar *et al.* (2004) reported the range of glucosinolates in rapeseed from 85-250 µmole/g defatted seed meal. Glucosinolates derived from amino acid

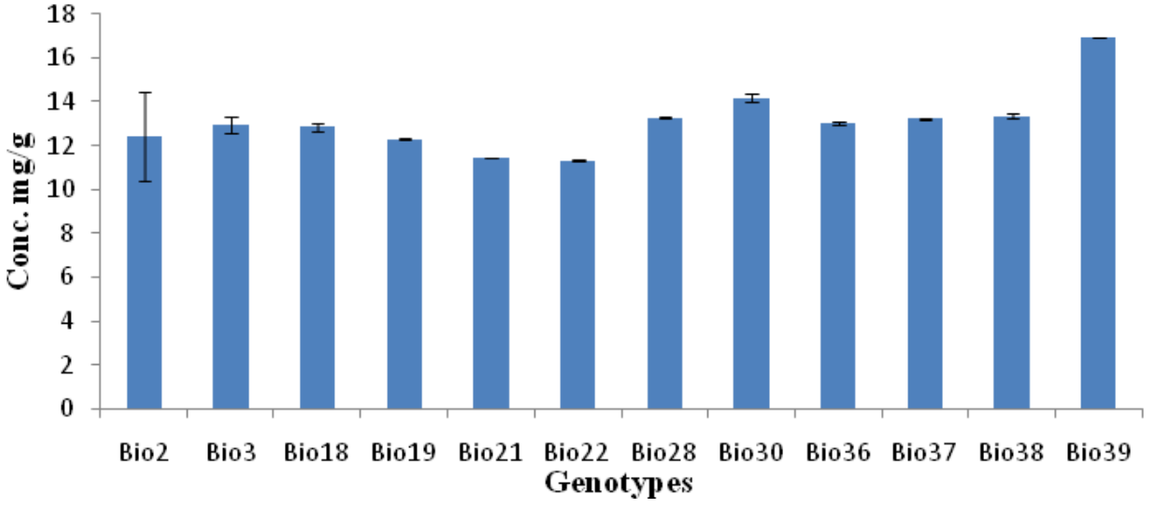

Figure 1: Total phenols content of defatted meal of some yellow-seeded Brassica genotype

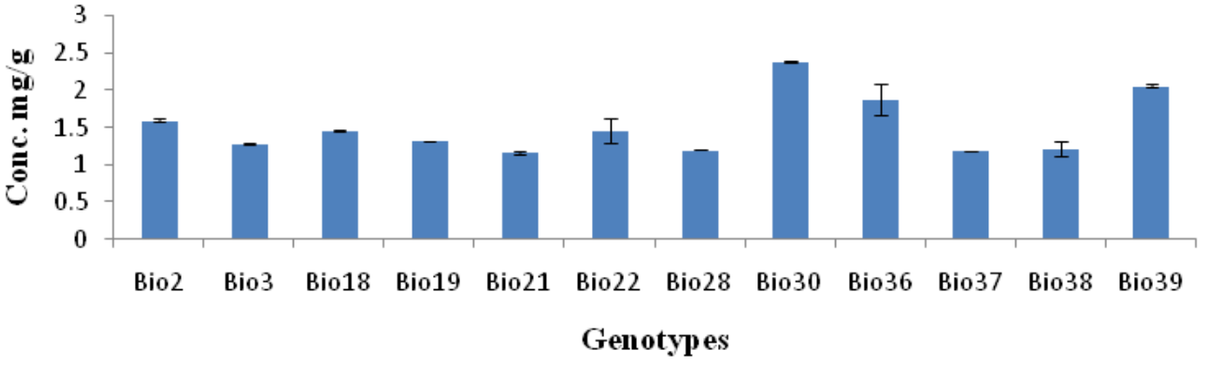

Figure 2: Ortho-dihydroxyphenols content of defatted meal of some yellow-seeded *Brassica* genotypes.

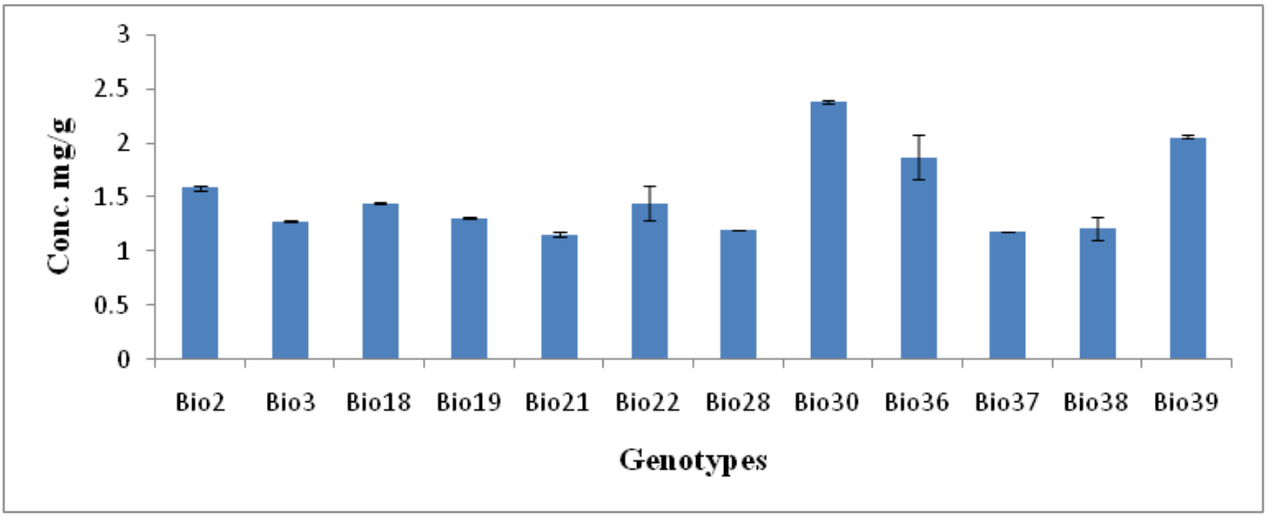

Figure 3: Total flavonoids content of defatted meal of some yellow-seeded *Brassica* genotypes.

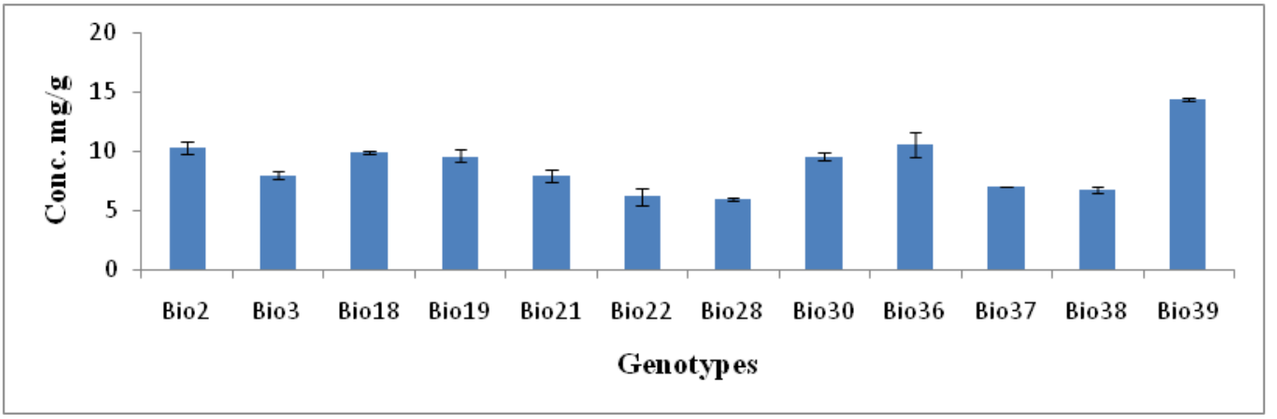

Figure 4: Total total antioxidants content of defatted meal of some yellow-seeded *Brassica* genotypes.

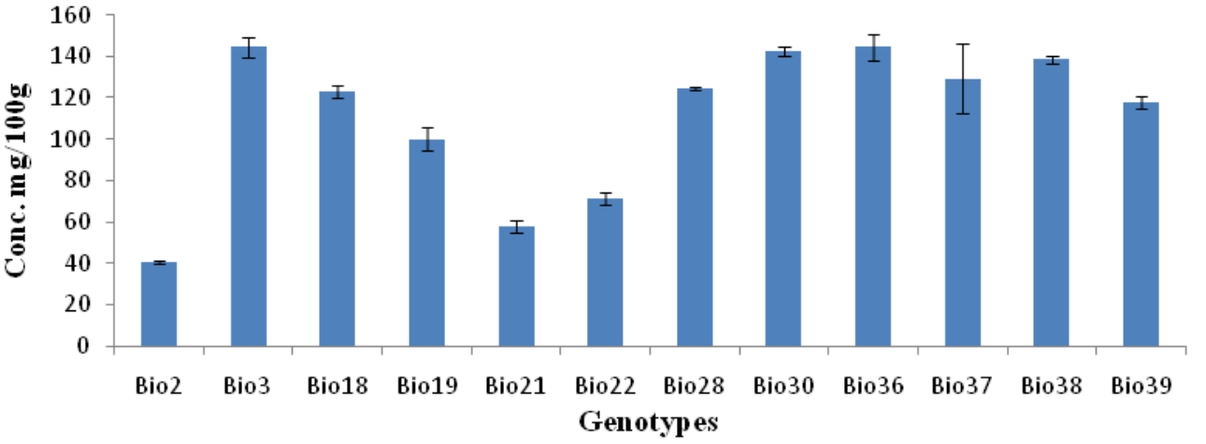

Figure 5: Vitamin C content of defatted meal of yellow-seeded *Brassica* genotypes.

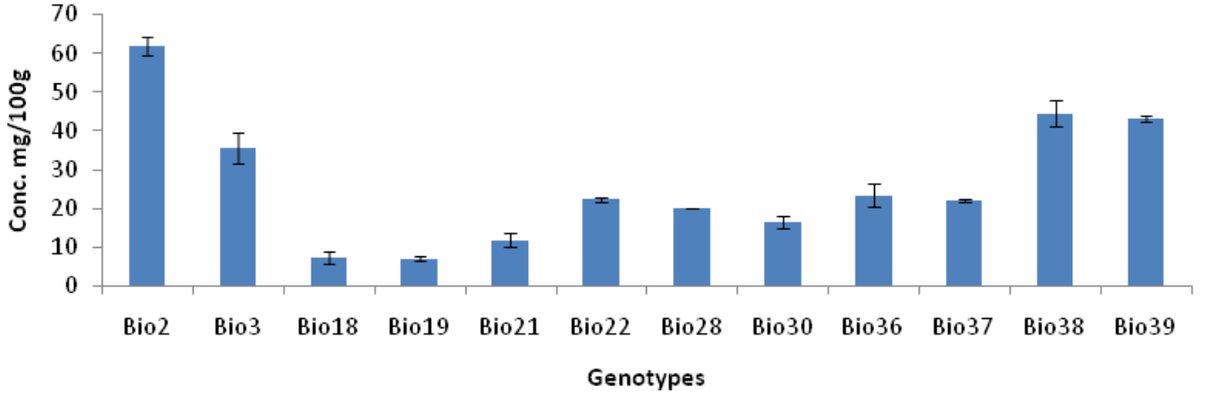

Figure 6. Total tocopherol content of defatted meal of some yellow-seeded *Brassica* genotypes.

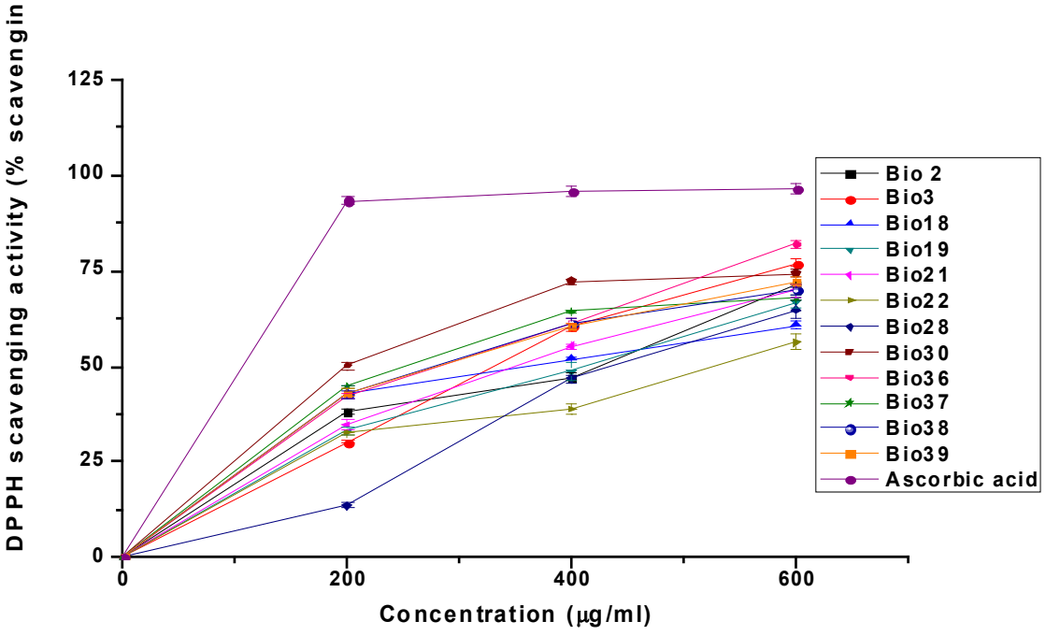

Figure 7. DPPH radical scavenging activity of defatted meal of yellow-seeded Brassica genotypes.

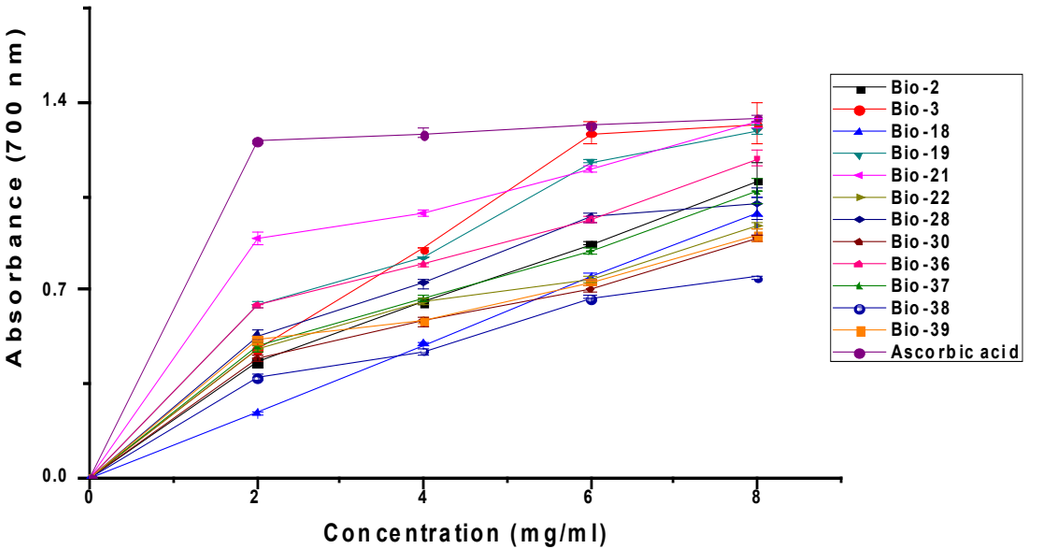

Figure 8: Reducing Power Activity of defatted meal of yellow-seeded *Brassica* genotypes.

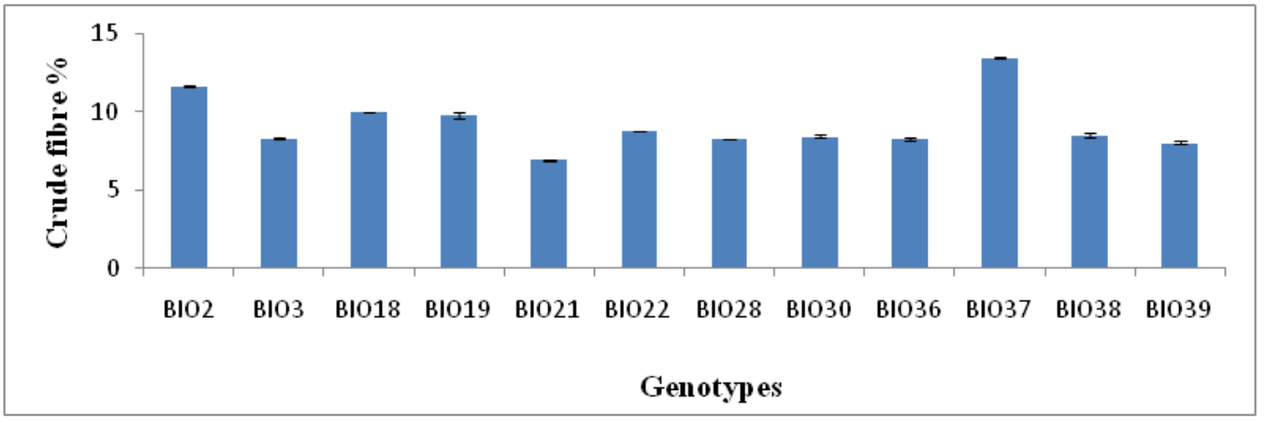

Figure 9: Total crude fibre content of defatted meal of some yellow-seeded *Brassica* genotypes.

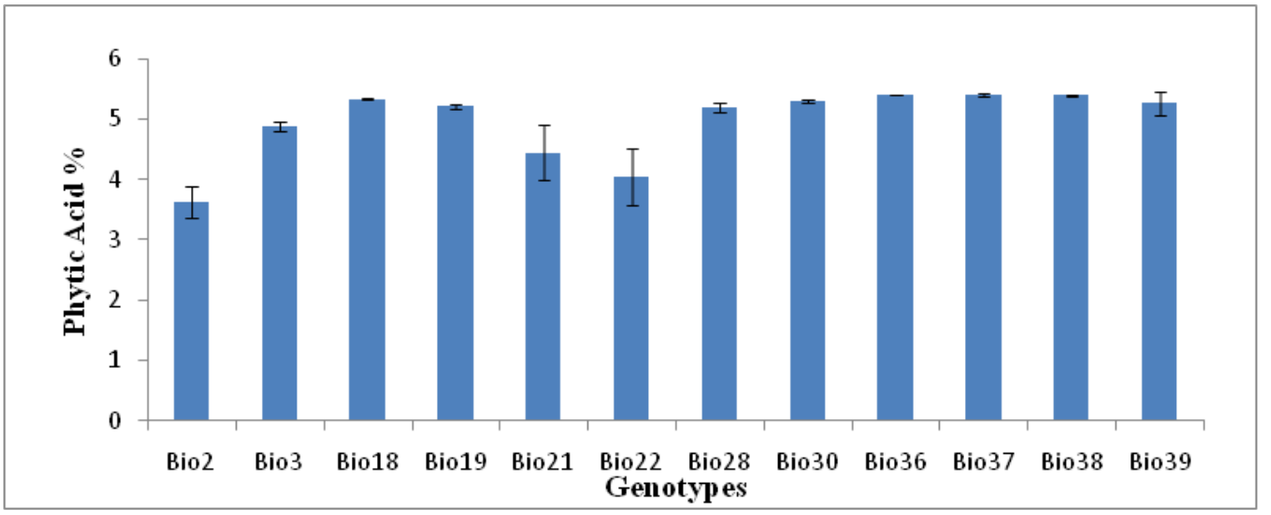

Figure 10: Total phytic acid content of defatted meal of some yellow-seeded *Brassica* genotypes.

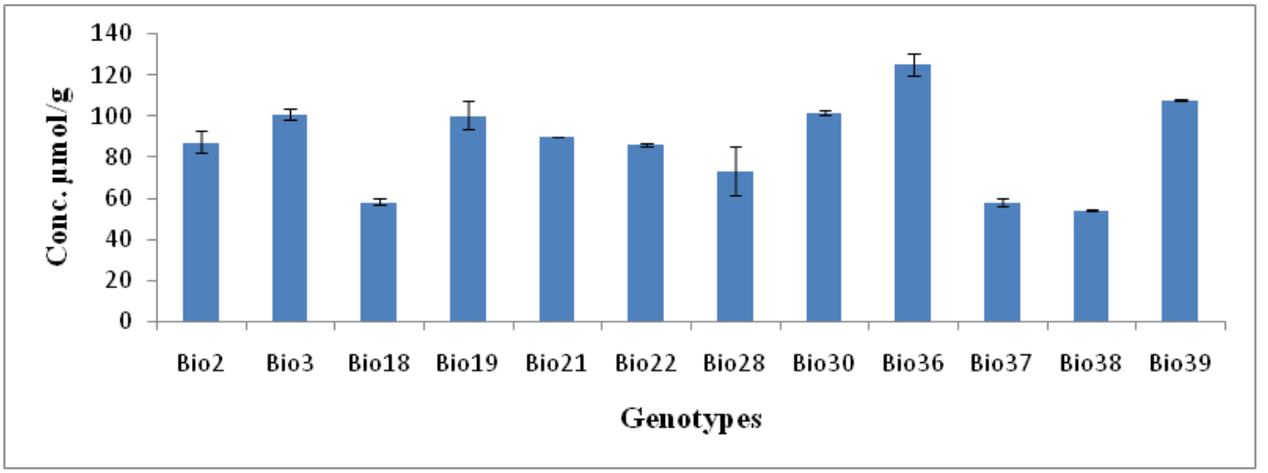

Figure 11: Glucosinolates content of defatted meal of some yellow-seeded *Brassica* genotypes.

biosynthesis and are important secondary metabolites in rapeseed-mustard, involved in plant defence against pests and diseases (Zrybko *et al.* 1997). The high oil content with low glucosinolate, low erucic and saturated fatty acid content, preferred for canola type varieties (Tahira *et al.* 2015). Glucosinolates together with phytic acid contribute to the anti-nutritional properties of rapeseed meal (Tripathi and Mishra, 2007). But, they are playing an important role in the prevention of cancer and other chronic and degenerative diseases (Fahey *et al.* 2003).

Conclusion

The genotypes of yellow-seeded *Brassica* revealed different levels of antioxidants based on their scavenging activity due to their hydroxyl groups. Because variation may arise due to various factors such as genotype, agronomic, environmental, developmental stage and post-harvest conditions. The antioxidative activities of the defatted meal determined by DPPH radical scavenging and reducing power activity. The defatted meal with the lower level of antinutrients but higher antioxidant levels can be utilized in the preparation of functional foods. The present study may useful for determining the quality and nutritional status of the genotypes, to ascertain their potential for development of new and better cultivar.

Acknowledgement

Authors are thankful to DES, Pantnagar and Dean CBSH for providing the necessary facilities for the execution of present research work.

References

Ahuja, KL, and Bajaj, KL 1999. Calorimetric determination of crude fibres in cruciferous oilseeds. *Cruciferae Newsletter* **21**: 61–62.

Backer H, Frank O, De Angells B and Feingold S 1980. Plasma tocopherol in man at various times after ingesting free or ocetylaned tocopherol. *Nutrition Reports International* **21**: 531–536.

Batista C, Barros L, Carvalho AM and Ferreira, ICFR 2011. Nutritional and nutraceutical potential of rape (Brassica napus L. var. napus) and ''tronchuda'' cabbage (Brassica oleraceae L. var. costata) inflorescences. *Food and Chemical Toxicology* **49**: 1208–1214.

Birt DF, Hendrich S and W Wang. 2001. Dietary agents in cancer prevention: Flavonoids and isoflavonoids. *Pharmacology & Therapeutics* **90**: 157–77.

Block G, C Jensen, M Dietrich, EP Norkus, M Hudes and L Packer. 2004. Plasma C-reactive protein concentrations in active and passive smokers: Influence of antioxidant supplementation. *Journal of the American College of Nutrition* **23**(2): 141–147.

Choi, YM, DO Noh, SY Cho, HJ Suh, KM Kim, and JM Kim. 2006. Antioxidant and antimicrobial activities of propolis from several regions of Korea. *LWT - Food Science and Technol* **39**: 756–761

Kaur, Dasmeet, Vishal Chugh, and Giridhar Soni. 2014. An in vitro and ex vivo study on antioxidant activity of Coriander Seeds. *International Journal of Agriculture, Environment and Biotechnology* **7**(3): 441-454.

Davey, MW, MVan Montagu, D Inze, M Sanmartin, A Kanellis, and N Smirnoff. 2000. Plant L-ascorbic acid: Chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture* **80**: 825–860.

Davies, NT, and H Reid. 1979. An evaluation of phytate, zinc, copper, iron and availability from soy based textured vegetable protein meat substitutes or meat extruders. *British Journal of Nutrition* **41**: 579–581.

De Nicola, GR, M Bagatta, E Pagnotta, D Angelino, L Gennari, P Ninfali, P Rollin and R Iori. 2013. Comparison of bioactive phytochemical content and release of isothiocyanates in selected Brassica sprouts. *Food Chemistry* **141**: 297–303.

Fahey, JW, KL Wade, KK Stephenson, and FE Chou. 2003. Separation and purification of glucosinolates from crude plant homogenates by high-speed counter-current chromatography. *Journal of Chromatography A* **996**: 85–93.

Funda, E A, and D Murat. 2011. Comparative analysis of vitamin C, crude protein, elemental nitrogen and mineral content of canola greens (Brassica napus L.) and kale (Brassica oleracea var. acephala). *African Journal of Biotechnology* **10**(83): 19385−19391.

Gupta, S, MK Sangha, G Kaur, AK Atwal, P Kaur, H Kumar, S Banga, and SS Banga. 2012. Variation in glucosinolate and tocopherol concentrations in a germplasm collection of Brassica juncea (L.) Czern. + Coss. *Biochem Anal Biochem* 1−7.

Hashmi, SI, PN Satwadhar, RR Khotpal, HW Deshpande, KA Syed, and BP Vibhute. 2010. Rapeseed meal nutraceuticals. *Journal of Oilseed Brassica* **1**(2): 43−54.

Hurrell, RF 2003. Influence of vegetable protein sources on trace element and mineral bioavailability. *Nutrition Journal* **133**: 2973S–2977S

Iqbal Muhammad, Iqbal Hussain, Asma Habib, Ashraf Muhammad Arslan and Rasheed Rizwan 2015. Effect of Semiarid Environment on Some Nutritional and Antinutritional Attributes of Calendula (Calendula officinalis). *Journal of Chemistry* Article ID 318506, 8pages http://dx.doi.org/10.1155/2015/318506.

Yue Jin, Shang Pingping, Wang Gang, Liu Dan, Xu Ling and Zhou Weijun 2014. Nutritional and Antioxidant Properties of Rapeseed (Brassica Napus) Cultivars with High and Low Erucic Acid Content. *Journal of Food and Nutrition Research* **2**(12): 918-924.

Kim S, and G Ishii 2007. Effect of storage temperature and duration on glucosinolate, total vitamin C and nitrate contents in rocket salad (Eruca sativa Mill.). *Journal of the Science of Food and Agriculture* **87**: 966−973.

Kumar S, SK Yadav, JS Chauhan, AK Singh, and NA Khan. 2004. Total glucosinolate estimation by complex formation between glucosinolates and tetrachloro palladate (II) using ELISA reader. *Journal of Food Science and Technology* **41**: 63–65.

Lai LS, Chou ST and Chao WW 2001. Studies on the antioxidative activities of Hsian-tsao (Mesona Procumbens Hemsl) leaf gum. *Journal of Agricultural and Food Chemistry* **49**: 963−968.

Law MY, Charles SA and Halliwell B 1983. Glutathione and ascorbic acid in spinach (Spinacia oleracea) chloroplasts. The effect of hydrogen peroxide and paraquat. *Biochemical Journal* **210**: 899–903.

Marina C, Zika L, Zoran Z, Senka V and Svetlana M 2009. Antioxidant properties of rapeseed. Zbornik radova Tehnološkog fakulteta u Leskovcu **19**: 27–33.

Morton LW, Caccetta RAA, Puddey IB and Croft KD 2000. Chemistry and Biological effects of dietary phenolic compounds: Relevance to cardiovascular disease. *Clinical and Experimental Pharmacology and Physiology* **27**: 152–59.

Pastuszewska B, Jablecki G, Buraczewska L, Dakowski P, Taciak M, Matyjek R and Ochtabinska A 2003. The protein value of differently processed rapeseed solvent meal and cake assessed by in vitro methods and in tests with rats. *Animal Feed Science and Technology* **106**: 175−188

Prieto P, Pineda M and Aguilar M 1999. Spectrophotometric quantitation of complex: specific application to the determination of vit E. *Analytical Biochemistry* **209**: 337–341.

Sasaki K and Takahashi T 2002. A flavonoid from Brassica rapa flower as UV-absorbing nectar guide. *Phytochemistry* **61**: 339–43.

Shahidi F, and Naczk M 1992. An overview of the phenolics of canola and rapeseed: chemical, sensory and nutritional implications. *Journal of American Oil Chemical Society* **69**: 917−924.

Singleton VL and Rossi JA 1965. Colorimetry of total phenolics with phosphomolybdic and phosphotungtic acid reagents. *American Journal of Enology and Viticulture* **16**: 144–158.

Tahira R, Ihsanullah AU Rehman, Saleem M 2015. Studies on Variability for Quality Traits, Association and Path Analysis in Raya (Brassica juncea) Germplasm. *International Journal of Agriculture and Biology* **17**: 381–386

Thanaseelaan V 2013. Proximate analysis, minerals and amino acid profiles of deoiled rapeseed meal. *International Journal of Food, Agriculture and Veterinary Sciences* **3**(1): 66–69.

Tripathi MK and Mishra AS 2007. Glucosinolates in animal nutrition: A review. *Animal Feed Science and Technology* **132**: 1−27.

Williams RJ, Spencer JPE and Rice EC 2004. Flavonoids: Antioxidants or signalling molecules? *Free Radical Biology and Medicine* **36**: 838–49.

Yen GC and Duh HY 1993. Antioxidative properties methanolic extracts from peanut hulls. *Journal of American Oil Chemist's Society* **70**: 383–386.

Zrybko CL, Fukuda EK, Rosen RT 1997. Determination of glucosinolates in domestic and wild mustard by high-performance liquid chromatography with confirmation by electrospray mass spectrometry and photodiode-array detection. *Journal of Chromatography A* **767**: 43–52.